

Studies of spontaneous activity with
a modified squirrel wheel technique
with special regards to screening and
follow-up-studies of sedative drugs

BY

SVEN STRÖM

STOCKHOLM 1964

Studies of spontaneous activity with a modified squirrel wheel technique

with special regards to screening
and follow-up-studies of sedative drugs

AKADEMISK AVHANDLING

SOM MED TILLSTÅND AV KUNGL. FARMACEUTISKA INSTITUTETS
LÄRARKOLLEGIUM FÖR ERNÅENDE AV FARMACIE DOKTORSGRAD
OFFENTLIGEN FÖRSVARAS I KUNGL. FARMACEUTISKA INSTITUTETS
FÖRELÄSNINGSSAL, KUNGSTENSGATAN 49,
DEN 27 MAJ 1964 KL. 9 F.M.

AV

SVEN STRÖM

FARM. LIC.

STOCKHOLM 1964

ACTA PHARMACEUTICA SUECICA

Volumen 1, Supplementum 1

Studies of spontaneous activity
with a modified squirrel wheel technique

with special regards to screening
and follow-up-studies of sedative drugs

by

SVEN STRÖM

STOCKHOLM 1964



Printed in Sweden

K L BECKMANS TRYCKERIER AB

CONTENTS

PREFACE.		5
INTRODUCTION.		7
CHAPTER I.	METHODS AND MATERIAL	
	1. Statistical Methods	12
	2. Experimental Animals	13
	3. Apparatus used	13
	4. Administration of Drugs	16
	5. Determination of letal Dose (LD_{50})	16
	6. Determination of hypnotic Dose (HD_{50})	17
	7. Potentiated Narcosis	17
CHAPTER II.	SOME FACTORS INFLUENCING THE VARIABILITY OF SPONTANEOUS ACTIVITY.	18
	<i>Discussion</i>	26
CHAPTER III.	SOME DRUGS INFLUENCING THE NORMAL SPON- TANEOUS ACTIVITY	32
	<i>Discussion</i>	73
CHAPTER IV.	ESTIMATION OF THE LOG DOSE RESPONSE LINE	82
	<i>Discussion</i>	91
CHAPTER V.	QUANTITATIVE STUDIES OF RELATIVE POTENCY	92
	<i>Discussion</i>	97
CHAPTER VI.	GENERAL DISCUSSION	99
SUMMARY		105
REFERENCES		108

PREFACE

The present investigation was carried out at the Pharmacodynamical and Toxicological Department of the Royal Pharmaceutical Institute and I want to express my sincere gratitude to its head Professor HÅKAN RYDIN for never-failing interest, useful advice and constant encouragement.

I am greatly indebted to Docent STELLAN LJUNGBERG who followed my work from the initial stages and has given me many valuable suggestions.

I take this opportunity of expressing my sincere thanks to Professor FINN SANDBERG and Docent DAVID INGVAR for stimulating discussions.

My thanks are also due to my colleagues at the Department for friendly help and I wish to express my particularly appreciation to Miss MONICA HELLQUIST for skilful technical assistance.

I offer my sincere thanks to Apotekarsocieteten for given me the opportunity to carry out this investigation.

I also wish to thank all the drug companies for their kindness in supplying me with the drugs used in this investigation.

Mrs EVA MARSHALL and Mr. P.E. BURKE translated the paper and I am grateful for their co-operation.

Stockholm, April 1964

Sven Ström

INTRODUCTION

NUMEROUS methods have been suggested to study the sedative effects of drugs in experiments on animals. However, up to now no completely satisfactory method has been found. The reason for this may probably be the difficulty in delimiting the measurable effect on animals from other effects which can be said to be sedative and which could influence the result, such as narcotic and toxic effects. The need for reliable methods in this field has been further increased by the introduction of tranquilizers and allied drugs.

This paper will deal with only some of the methods which are used to test the sedative effect. For a more comprehensive survey of the field, reference is made to RILEY & SPINKS (1).

Potentiated narcosis as a test of a sedative effect was introduced by WINTER (2). This was for the determination of the duration of a standard hypnotic by itself or in combination with the substance to be investigated. All central depressant drugs probably give a potentiated narcosis, but so do many other substances, such as adrenaline, serotonin, histamine and glucose. The method should be suitable as a rough screening-test as it is simple and can be carried out as a matter of routine. However, the test should preferably be supplemented by a more selective method for determining whether there is a sedative effect.

Other criteria to determine a sedative effect are hypothermia and reduced metabolism (3, 4, 5, 6). With both chlorpromazine and reserpine LESSIN & PARKES (3) found a good relationship between the reduction of the spontaneous activity, determined by the photo-electric method (see below) and the lowering of the body temperature of the experimental animals.

The determination of the "fall-time" of mice on a sloping mesh was used by THOMPSON (7) for the testing of insulin. By "fall-time" is meant the period of time the animals are capable of remaining on the mesh before they fall off. Modifications of this method have been used for determining the sedative effect (8). Here a fixed (immovable) apparatus has been used. It is also possible to use a moveable apparatus to determine how long the animals can stay on the apparatus before and after the administration of the substance in question. This group includes the rotating rods (9, 10, 11) with which TRIPOD & GROSS (9) have demonstrated that on calculating the all-or-none effect some substances with sedative effect show a linear dose effect curve.

A climbing test used by KNEIP (12) is based on the fact that a number of mice in a cage normally climb up an inclined ladder within ten minutes.

After administering a central depressant drug, only, a few or none of the animals — depending on the amount of the dose — will climb up the ladder. This test also gives a certain indication of the initiative abilities of the animals.

By inducing hyperactivity (13, 14) on experimental animals with analectics such as amphetamine, mescaline, etc. and later determine the subduing effect of various sedatives, it is possible to obtain an idea of their effect. In such experiments the activity of the animals is studied often using apparatus, which will later be discussed in connection with the determination of the spontaneous activity.

Other methods of observing the central depressive effect is to investigate whether the substance has an anti-convulsant effect on electric shock, strychnine, picrotoxin, or other substances which may induce convulsions (15, 16).

Registration of the spontaneous activity in experimental animals must be regarded as the most used method of procedure when it is a question of observing the sedative effect of a substance. As a modification of this method has been used in this investigation it will be discussed here in more detail.

SZYMANSKI (17) devised an apparatus of the "jiggle cage" type. The principle on which he based this is that the cage, in which the experimental animal is placed, is suspended so that the movements of the animal are transmitted to the cage. Via an airdrum and Marey's capsule the registration could be made on a kymograph. Mice and salamanders were used as experimental animals. The apparatus and registration later took different forms (18, 19, 20, 21). This method has also been used by SANDBERG (22) for the quantitative determinations of phenothiazine derivatives.

Various designs of the fixed apparatus have been applied, but the latest and now most widely used is the photo-electric method introduced by SIEGEL (23). Every time the animals break a photo-electric beam, this activates a counter or recording instrument and so registers the activity of the animals. Later many have applied the same principle (3, 24, 25, 26, 27). SIEGEL has also mentioned infra-red rays for registering activity in the dark; this is probably very important as rats and mice are the animals most used for such experiments and the greatest activity of such animals is during the hours of darkness.

Squirrel wheels and rotating drums were first described by STEWART (28). This apparatus has later taken many different forms. The activity can be registered either by means of a kymograph (28, 29, 30, 31), mechanically (30, 31, 32, 33, 34, 35, 36) or electrically (37). Methods of estimating

the reliability of the squirrel wheels and of calibrating them, have also been described by SHIRLEY (38), LACEY (39) and others.

Another way of measuring the spontaneous activity is to have the animals move about on smoked paper, and thus themselves register their activity (40).

A mouse can also be placed on dry sand on a gauze, so that when the mouse moves the sand runs through the gauze and is collected and measured. The amount of sand is in proportion to the movements of the mouse (41). A further way of measuring the spontaneous activity is to use a tip-plate, which has been described by FORST (42).

In order to determine the sedative effect on animals it may be necessary to use several different methods. These should be well tested, both for variations of the method after administration of placebos and after giving known drugs.

It seems to me to be important that a method should be developed more thoroughly — not only qualitatively but also quantitatively — in order to allow a more reliable assessment of the results obtained. This can only be done by carefully studying the effects of known drugs and then relate the findings to the effect of new and unknown substances.

A survey article by LAURENCE (43) contains the following statement about experiments on animals with drugs:

"In the development of understanding of how drugs work and of what they can do, knowledge of which is an essential preliminary to the rational development of new drugs, it is still as important to do experiments on animals with drugs that have been found to be effective on man, as it is to do the reverse. In this way a body of knowledge of what can and what cannot be expected of animal tests for drugs may be built up."

Statistical methods have been used in recent years and this has created better possibilities for the assessment of various methods. Statistical processing also allows a quantitative evaluation of the effects of drugs in addition to determining the degree of reliability. Mathematical calculation of the relation between dose and effect gives considerably better information about the drug than qualitative studies; often a quantitative investigation can also provide a certain idea of the working of the drug in relation to other effects, such as toxic, as well as to other drugs.

This paper contains an account of the studies of the spontaneous activity of mice according to a modified method of the squirrel wheel technique using an apparatus designed by LJUNGBERG (37).

For several reasons I have decided to make a more thorough study of this apparatus.

As has been mentioned earlier, the study of the spontaneous activity is one of the most common methods to find out objectively the sedative effect on animals. It is especially advantageous to use mice as experimental animals as both where it is a question of pharmacological screening and possible continuous quantitative studies generally very large number of animals are required. Mice are comparatively cheap experimental animals, as well as being easier and quicker to work with than larger animals. In addition to providing a measure of the spontaneous activity, the apparatus used here should give an idea of the animals' ability to take initiative as well as of their ability to co-ordinate their movements. This apparatus differs in effect from the most commonly used squirrel wheel in that the animals do not necessarily have to be inside the wheel but have the possibility of jumping into or out of the wheel as they wish, as they have a large space in which to run about inside the cage (cf. Figure 1). An idea of the behaviour pattern of the animals can thus be obtained.

During the period of the experiment the mice are not subjected to outside pressure or influence of the surroundings; this is considered a desirable factor in experiments of this type. Another reason for my decision to study this technique for the determination of spontaneous activity is that LJUNGBERG (37) in his account of the technique has shown that for phenobarbital sodium there is a connection between dose and effect, which can be demonstrated in a linear dose-effect curve. The dose-effect relationship can thus be statistically analysed.

The aim of this paper is to investigate the suitability of squirrel wheel technique for the study of substances having a supposed central depressive effect both for screening purpose and for quantitative studies. Here it is desirable that the following criteria should be fulfilled:

The experimental animals should have a spontaneous activity suitable for experimentation;

the method should be sufficiently accurate;

the method should be sufficiently sensitive so that the registered effects can be delimited from other pharmacological effects with the substances; and, finally,

the same apparatus should be usable for continued studies of the sedative effect on the substances, which have been found to be active in the screening tests.

In the investigation studies have been made of the time the mice took to reach constant measurement values after having been placed in the test

apparatus in a darkened room and in this connection the period for altering the daily rhythm of the animals. In fact, the studies of the sedative effects should be made during the animals' active period, which in the case of mice is the period of darkness. The altering of the daily rhythm of the animals has been done for practical reasons, as their active period, i. e. the test period, will then be the same as the working hours at the laboratory as this facilitates the work.

In the above-mentioned work on phenobarbital sodium with this apparatus (37), two mice were put in each cage. Thus I considered that it was of interest to study both the difference in the relative standard deviations and the sensitivity of the method for different drugs when one or two animals were placed in each cage.

Furthermore, the lethal mean dose (LD_{50}) and the hypnotic mean dose (HD_{50}) have been determined for mice of the same breed and sex, which were used for determining the spontaneous activity with the substances, where these effects can be considered to influence the investigations. As potentiated narcosis is one of the simplest and generally the first method to be used to get an idea of a sedative effect, I have considered it to be of interest to study this method at the same time as the spontaneous activity at corresponding dose levels.

Finally, dose-effect studies and comparative quantitative determinations for certain drugs have been made.

Altogether, some thirty known drugs have been studied in various respects, some 6 000 mice have been used, and about 70 000 measurements have been registered and form the basis for the assessments which have been made.

CHAPTER I

METHODS AND MATERIAL

1. Statistical Methods

The statistical methods used were taken from BLISS (44), BURN (45), FINNEY (46), FISHER & YATES (47), GADDUM (48), LJUNGBERG (49) and PHARMACOPOEA NORDICA VOL. IV (50).

The symbols used are identical with those in Pharmacopoea Nordica.

The *standard deviation* (s) was calculated from the formula:

$$s = \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

The *standard error of the mean* ($S.E.$) was calculated as:

$$S.E. = \pm \sqrt{\frac{s^2}{n}}$$

The *relative standard deviation* ($s_{\%}$) was used as an expression of the standard deviation in comparative experiments and calculated as:

$$s_{\%} = \frac{s \cdot 100}{\bar{x}}$$

In calculating the difference between two mean values, the standard error of the difference (s_d) was determined as the square root of the sum of the corrected mean deviations of the two samples:

$$s_d = \pm \sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}$$

in which n_A and n_B are the number of values included in determination of A and B , respectively.

Student's t test was used to determine whether a difference is true or is only due to chance:

$$t = \frac{d}{s_d}$$

The probability level $P = 0.05$ has then been used, as when giving the fiducial limits:

$$\bar{x} - t \cdot s_{\bar{x}} \text{ and } \bar{x} + t \cdot s_{\bar{x}}, \text{ respectively.}$$

In calculating the *regression lines*, the slope (b) was calculated as:

$$b = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2}$$

and its standard error:

$$s_b = \pm \sqrt{\frac{S(y - \bar{y})^2 - \frac{[S(x - \bar{x})(y - \bar{y})]^2}{S(x - \bar{x})^2}}{(n - 2) \cdot S(x - \bar{x})^2}}$$

In the *analysis of variance*, s was calculated as the square root of the mean square of the residual variation, after segregation of the known causes of variation.

According to GADDUM (48) the *index of precision* was calculated as:

$$\lambda = \frac{s}{b}$$

In comparison of variances the probability level $P = 0.05$ has been used as the limit of significance.

2. Experimental Animals

Male albino mice weighing between 18 and 23 g from the same animal supplier have been used as experimental animals for determining both the spontaneous activity, LD_{50} , HD_{50} , and potentiated narcosis. During the investigation of the spontaneous activity the animals were given a standard diet and water *ad libitum*. The standard diet consisted of "mouse bread", containing powdered milk, bran, meat, bone-meal and fish-meal, as well as standardized quantities of vitamins A, B, D and E.

The feeding of the animals used in the various experiments took place once a day between 3 p.m. and 4 p.m.

3. Apparatus used for Determination of Spontaneous Activity

For registered spontaneous activity has been used an apparatus previously described by LJUNGBERG (37).

A squirrel wheel was placed in a cage measuring about 40 by 22 by 22 cm against one of the short walls; the wheel was 15 cm wide and 15 cm in diameter, and the rungs 2 cm apart. The axis of the wheel was attached to the wall by a wall bracket and an eccentric cam actuating an electric contact was placed at the free end of the axis. Each revolution of the wheel thus produced an electric impulse, which was transmitted to an electro-mechanical comptometer (Figures 1 and 2).



Fig. 1. In each cage there is a squirrel wheel and a small house. Each revolution of the wheel produces an electric impulse, which is transmitted to an electro-mechanical comptometer.

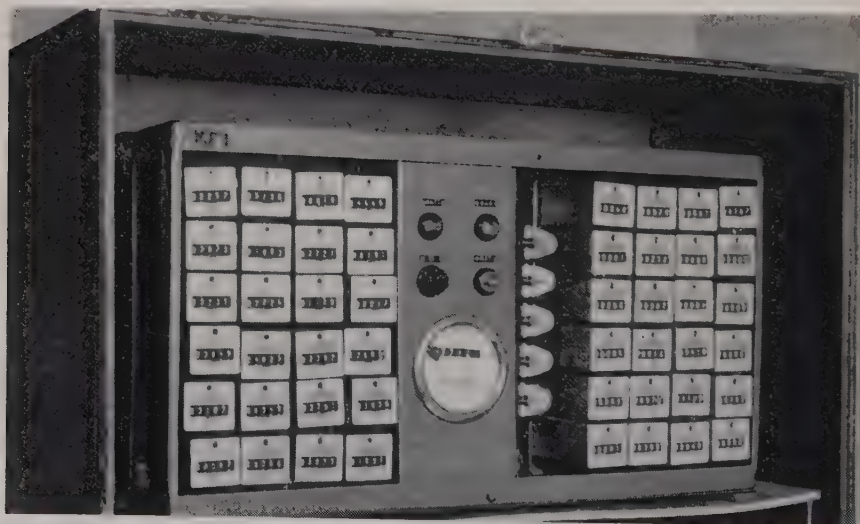


Fig. 2. The comptometers are brought together into a single units which is placed in another room so that readings can be taken without disturbing the animals being tested.

Each revolution was registered electrically with two comptometers for each wheel; one was for registering diurnal activity and the other nocturnal activity. The comptometers were brought together into a single unit which was placed in another room so that readings could be taken without disturbing the animals being tested. As can be seen from Figure 1, the animals in the cages can jump in and out of the squirrel wheels on their own initiative, and can run around in them as they will. For each experiment 24—30 such cages were used. Different experiments were carried out having one or two animals in each cage. For such purposes each mouse or pair of mice was kept all the time in the same cage and used the same squirrel wheel. The temperature in the room where the experiments were undertaken was kept between 20° C and 23° C. In order that the active period of the animals, which is during the hours of darkness, should coincide with the working hours of the laboratory, the light was put out at 9 a.m. and put on at 9 p.m. Simultaneously the electrical impulses from the wheels were switched from the night to the day comptometers and vice versa. All studies started at 9 a.m.

The comptometers were read every half-hour during the five hour period after administration and in the same order as the administration, which levels out the time differences to a certain extent. In addition, both the total period of activity and the total period of rest were noted. During the later part of the investigation the comptometers were recorded by means of an automatic camera, and the film negative was read by means of an enlarger. For practical reasons the average number of revolutions for placebo and the test-substance has formed the basis for the assessment, as a rule, in the screening-tests mentioned in Chapter III. However, the readings were taken for each individual squirrel wheel to allow statistical calculations to be made where this was considered necessary. As can be seen from Chapter III, a Student analysis has also been carried out in certain cases to determine the differences between test preparation and placebo.

In the dose-effect studies the experimental animals were divided up into equally large groups according to the number of doses. One dose was given every day, and all the doses were administered to all the animals in a random order. By this means the length of the test period was determined by the number of doses. Other quantitative determinations have been made as 2×2 factor analysis, i.e. as a comparison between two different drugs on two dosage levels. Consequently, here the animals were divided into four groups and all the groups were given both high and low doses of both the preparations.

4. Administration of Drugs

In all the experiments, with the exception of the dose-effect studies on serotonin and hexobarbital sodium in potentiated narcosis which were given intraperitoneally, the preparations were given *per os*, as this type of administration is the most commonly used in therapy where drugs of this nature are involved.

For this type of administration was used a specially designed cannula which had been bent at an angle of approx. 135° (Figure 3).

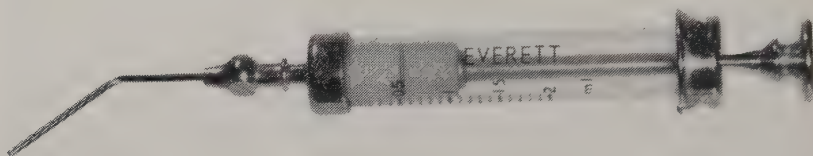


Fig. 3. For orally administration was used a specially designed cannula, which had been bent at an angle.

For determining the spontaneous activity both placebo (physiological saline solution) and the preparation were given *per os* between 8.45 a.m. and 9 a.m. Where possible the preparation was administered dissolved in water, while drugs insoluble in water were given suspended in a 0.1 per cent solution of ethyloxyethylcellulose (Modocoll® E 400). The volume administered was always 0.5 ml an animal, and the dosage was calculated on the mean weight of the mice used for the experiment.

The investigations of certain drugs on spontaneous activity (Chapter III) were done as a cross-over test using physiological saline solution as placebo, after which the activity was expressed as a percentage of the placebo.

5. Determination of the Lethal Dose (LD_{50})

In order to obtain an approximate comparison between toxic dose and test dose preliminary toxicity calculations were made for all preparations with LD_{50} less than 1 000 mg/kg. In these cases LD_{50} was calculated using probit assay, and at least 30 mice were used for each calculation. The administration was *per os* and the drug was administered dissolved in water or suspended in an 0.1 per cent solution of ethyloxyethylcellulose.

6. Determination of Hypnotic Dose (HD_{50})

The criterium to be used in a quantal response for the determination of HD_{50} can obviously be of great importance in the interpretation of results obtained from different preparations. Here loss of righting reflex for at least 30 seconds has been chosen. This preliminary determination of HD_{50} was intended only to supplement the determination of the spontaneous activity. HD_{50} was calculated by means of probit assay. At least 30 mice were used for each determination and the administration was *per os*.

7. Potentiated Narcosis

Potentiated narcosis has been determined in principle by WINTER (2). The test preparation was given to a group of ten mice orally, and after 30 minutes 100 mg/kg of hexobarbital sodium was injected intraperitoneally. At the same time a control group was given only 100 mg/kg hexobarbital sodium intraperitoneally. The individual periods of sleep were noted and possible differences between test group and control group was then determined by means of Student analysis. As was the case with the HD_{50} determinations, loss of righting reflex for at least 30 seconds was taken as a criterium of hypnosis.

CHAPTER II

SOME FACTORS INFLUENCING THE VARIABILITY OF SPONTANEOUS ACTIVITY

THE experiments involving spontaneous activity were carried out in the dark, which is the active period for mice. In order that the test period should coincide with the working hours of the laboratory, the experiments were made in a darkened room, which has been described earlier. Consequently, the mice needed a certain time to adapt themselves to the new daily rhythm and some additional time to learn how to run in the wheels and get used to their new surroundings.

In order to ascertain the time taken for the mice to attain a relatively constant number of revolutions of the wheel from day to day, if the animals had not previously been kept in a darkened room, two mice from a group were put into each cage. The comptometers were read after five hours and also after the total period of activity and the total period of rest. The period of the experiments totalled 25 days, during which time the mice were given neither placebo nor drugs. The results are shown in Figure 4 and Table 1. During the test period, which obviously is of the greatest interest in this connection, the number of revolutions was considered to have reached a stable level on the tenth day (Figure 4). From this the regression line for the days which followed was plotted and the individual values for each cage were used as a basis for the calculation. As can be seen from the values in Table 1, no deviation from the regression line could be noted. The table also shows that the line is not inclined in any specific direction.

Table 1. Test of linearity for the mean values of the test period after the mice had reached a stable spontaneous activity. Two mice per cage. Cf. Figure 4.

	<i>df</i>	Sum of squares	Mean square
Regression	1	22 583 160	22 583 160
Departure from regression	14	126 103 205	9 007 371
Between days	15	148 686 365	9 912 424
Error	402	3 220 700 659	8 011 693
Total	417	3 369 387 024	

Slope of regression line: $b = 42.36 \pm 29.46$ $t = 1.675$ $P > 0.05$

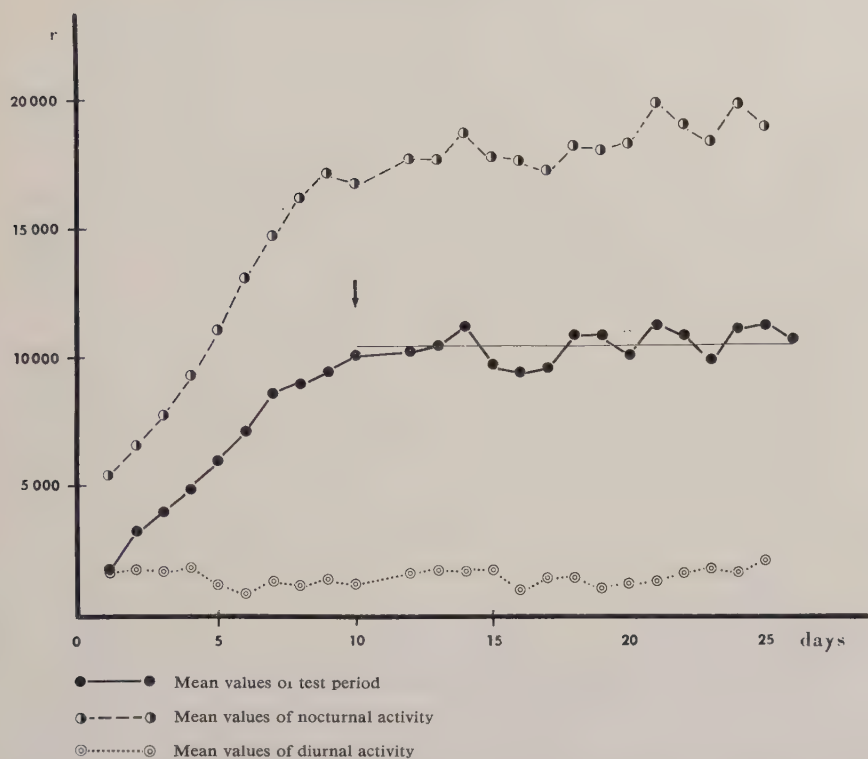


Fig. 4. Spontaneous activity during 25 days for *two mice per cage*, where the animals had not previously been trained and adapted to the new daily rhythm. The animals were given neither placebo nor drugs. Each point is the mean of about 25 values.

If the animals previously during a period of a fortnight had been allowed to adapt their daily rhythm by being kept in the same room as the apparatus used for the experiment, only six days seemed to be necessary for their spontaneous activity to attain a constant level, i.e. to get used to the apparatus (Figure 5 and Table 2).

Table 2 shows no deviation from the straight line. However, the regression line shows a slight but significant slope corresponding to an increase in spontaneous activity of $0.48\% \pm 0.24\%$.

The same experiment was carried out with mice, which had adapted their daily rhythm, having *one* mouse in each cage. The results are shown in Figure 6 and Table 3. Here also there was no deviation from the straight line, nor was there any definite inclination of the line. Five days were required in this case for the animals to attain an even spontaneous activity.

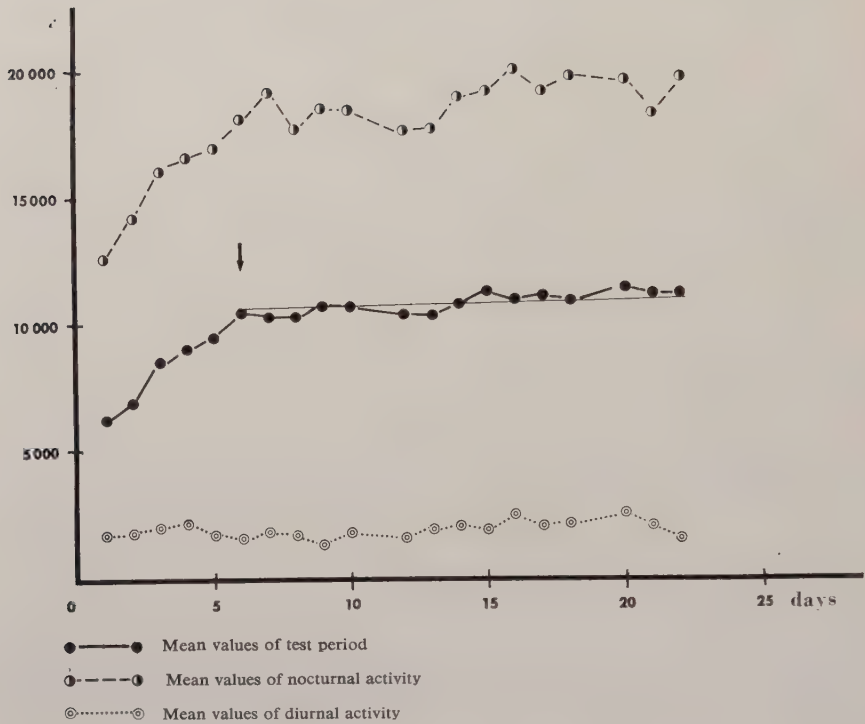


Fig. 5. Spontaneous activity during 22 days for two mice per cage, where the animals had not been trained but adapted to the new daily rhythm. The animals were given neither placebo nor drugs. Each point is the mean of about 25 values.

Table 2. Test of linearity for the mean values of the test period after the mice had reached a stable spontaneous activity. Two mice per cage. Cf. Figure 5.

	df	Sum of squares	Mean square
Regression	1	24 343 299	24 343 299
Departure from regression	13	15 382 619	1 183 278
Between days	14	39 725 918	2 837 566
Error	358	2 271 065 002	6 343 756
Total	372	2 310 790 920	

Slope of regression line: $b = 51.78 \pm 26.05$ $t = 1.987$ $P = 0.05-0.02$

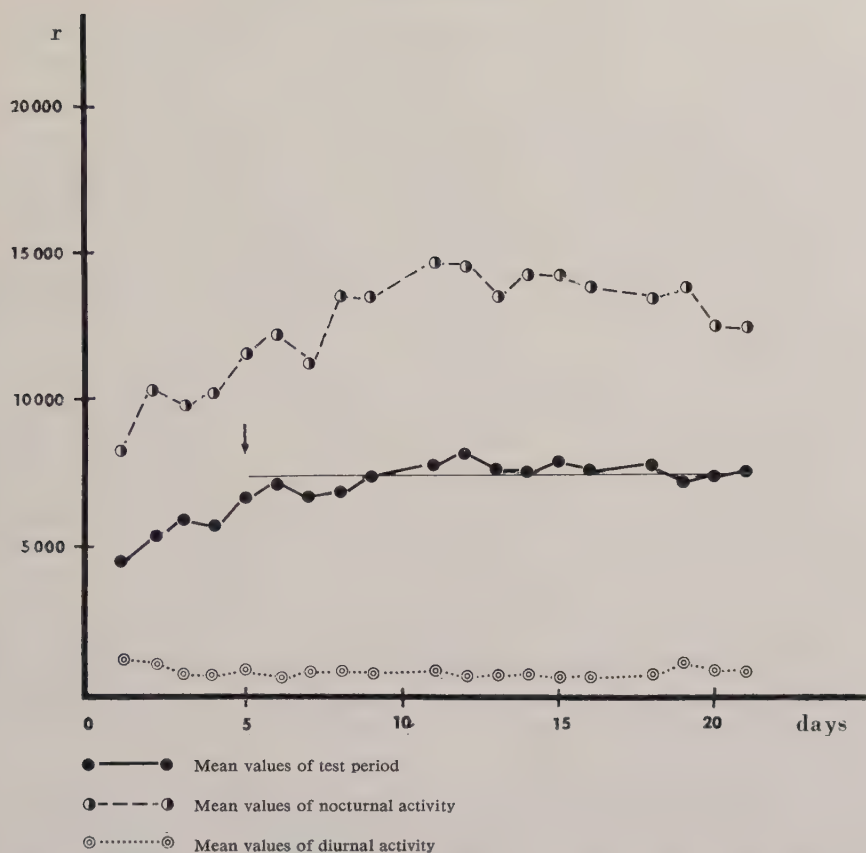


Fig. 6. Spontaneous activity during 21 days for *one mouse per cage*, where the animals had not been trained but adapted to the new daily rhythm. The animals were given neither placebo nor drugs. Each point is the mean of about 25 values.

Table 3. Test of linearity for the mean values of the test period after the mice had reached a stable spontaneous activity. *One mouse per cage. Cf. Figure 6.*

	<i>df</i>	Sum of squares	Mean square
Regression	1	21 722 542	21 722 542
Departure from regression	13	50 434 059	3 879 543
Between days	14	72 156 601	5 154 043
Error	377	5 351 212 655	14 194 198
Total	391	5 423 369 256	

Slope of regression line: $b = 46.82 \pm 37.39$ $t = 1.252$ $P > 0.2$

Figures 4, 5 and 6 show that the diurnal activity was fairly constant right from the start and that it was far less than the nocturnal activity both with one or two animals in the cage.

In order to get an idea of the relative deviation of the spontaneous activity of untreated mice, which had become used to the darkness and the apparatus, the standard deviation was reckoned as a percentage of the mean value of the total values for the entire apparatus from day to day (Table 4). Calculations were made both with one or two animals in a cage after the animals had attained an even spontaneous activity, i.e. from the tenth or the sixth day, respectively.

The mean values for the test period after the mice had attained an even spontaneous activity in the experiment with two animals per cage were $10\,561 \pm 153$ and $10\,692 \pm 87$, respectively. The corresponding mean values for the entire period of nocturnal activity were $18\,474 \pm 204$ and $18\,582 \pm 294$, respectively and for diurnal activity $1\,482 \pm 84$ and $1\,803 \pm 82$, respectively. In the regression calculations mean values were $10\,526$ and $10\,700$ respectively for the testperiod.

Table 4. Spontaneous activity for untreated mice which had been trained and adapted for the new daily rhythm. Cf. Figures 4 and 6.

1 mouse/cage	Test period	Nocturnal period	Diurnal period
Mean rev./cage and day $\pm$ S.E.	$7\,458 \pm 124$	$13\,452 \pm 267$	716 ± 39
S% $n = 15$	6.4 ± 1.2	7.7 ± 1.4	21.2 ± 3.9
2 mice/cage			
Mean rev./cage and day $\pm$ S.E.	$10\,561 \pm 153$	$18\,474 \pm 204$	$1\,482 \pm 84$
S% $n = 15$	5.8 ± 1.0	4.3 ± 0.8	21.9 ± 4.0

The variations between days and between cages for mice which had been adapted and trained for a fortnight were studied after administration of placebo by giving the mice saline orally for a five-day period, after which the spontaneous activity was registered every hour. The experiments were undertaken both with one or two animals in a cage and occupied the first five hours of the active period of the mice, i.e. which has been described earlier as the test period. In the statistical calculations where the aggregate values have been used (Tables 5 and 6) no variation *between* days could

Table 5. Variance analysis of aggregate values for spontaneous activity for a five-hour test period during five days with two mice in each cage.

	Hour	df	Sum of squares	Mean square	Variance ratio	P
Between cages	1	21	13 514 848	643 564	3.47	< 0.01
Between days		4	1 353 398	338 350	1.82	> 0.05
Error		84	15 582 919	185 511		
Total		109	30 451 165	$S = 430.69$	$S\% = 34.3 \pm 2.31$	
Between cages	2	21	59 165 338	2 817 397	5.15	< 0.01
Between days		4	1 874 541	468 635	1.17	> 0.05
Error		84	45 952 115	547 049		
Total		109	106 991 994	$S = 739.62$	$S\% = 28.4 \pm 1.92$	
Between cages	3	21	153 115 166	7 291 198	6.19	< 0.01
Between days		4	1 554 509	388 627	3.03	> 0.05
Error		84	98 928 335	1 177 718		
Total		109	253 598 010	$S = 1 085.25$	$S\% = 27.8 \pm 1.87$	
Between cages	4	21	261 519 660	12 453 317	5.81	< 0.01
Between days		4	5 337 183	1 334 296	1.61	> 0.05
Error		84	180 091 141	2 143 942		
Total		109	446 947 984	$S = 1 464.22$	$S\% = 29.4 \pm 1.98$	
Between cages	5	21	390 446 165	18 592 674	5.63	< 0.01
Between days		4	9 154 876	2 288 719	1.44	> 0.05
Error		84	277 315 708	3 301 377		
Total		109	676 916 749	$S = 1 816.97$	$S\% = 30.9 \pm 2.08$	

be demonstrated at any time of registration whether the cage contained one or two animals. On the other hand, the analysis showed a significant variation *between cages* at each reading time.

Naturally, the variation between cages might have been caused by greater sluggishness in one wheel than in the other. Thus animals who showed a low spontaneous activity were placed in other cages where the animals had shown lively activity. However, it appeared that the animals concerned showed the same activity pattern, whether it was low or high. Neither when new mice were brought in could it be shown that any specific comptometer had constantly low readings. The sluggishness of the wheel, therefore, probably played only a minor role as compared with the variations in the spontaneous activity of the different mice.

As has been mentioned earlier (Chapter I), the mean values of all the readings at each reading time formed the basis for assessing the results of the effect of the drug. Consequently, it has been important to determine the deviation for the entire apparatus from day to day at each reading time.

Table 6. Variance analysis of aggregate values for spontaneous activity for a five hour test period during five days with one mouse in each cage.

	Hour	df	Sum of squares	Mean square	Variance ratio	P
Between cages		26	36 654 311	1 409 781	16.7	< 0.01
Between days	1	4	420 118	105 030	1.25	> 0.05
Error		104	8 762 893	84 259		
Total		134	45 837 322	$S = 290.27$	$s\% = 31.8 \pm 1.94$	
Between cages		26	179 444 256	6 901 702	28.41	< 0.01
Between days	2	4	935 695	233 924	1.04	> 0.05
Error		104	25 266 559	242 948		
Total		134	205 646 510	$S = 492.88$	$s\% = 25.6 \pm 1.56$	
Between cages		26	474 740 529	18 259 251	33.20	< 0.01
Between days	3	4	2 947 553	736 888	1.34	> 0.05
Error		104	57 199 637	549 997		
Total		134	534 887 719	$S = 741.62$	$s\% = 26.0 \pm 1.58$	
Between cages		26	853 915 488	32 842 903	41.10	< 0.01
Between days	4	4	4 107 219	1 026 805	1.29	> 0.05
Error		104	83 100 271	799 041		
Total		134	941 122 978	$S = 893.89$	$s\% = 24.2 \pm 1.47$	
Between cages		26	1 313 848 577	50 532 638	43.46	< 0.01
Between days	5	4	4 976 366	1 244 092	1.07	> 0.05
Error		104	120 936 856	1 162 854		
Total		134	1 439 761 799	$S = 1 078.35$	$s\% = 24.1 \pm 1.47$	

In order to measure this deviation, placebo was given over a period of fifteen days. Also in this case the experiments were carried out both with one or two animals in a cage. Table 7 shows the deviation in percentage terms. With one mouse per cage also the 30-minute value was determined, which was shown to be 375 ± 18 with $s\% = 18.1\%$.

It should be noted that each mean value used as a base for the calculations comes from about 25 individual values. As can be seen, the relative deviation ($s\%$) is somewhat greater for one animal except for the fifth hour. However, no statistical difference could be shown between the variations for one or two animals at any of the five readings.

Nocturnal activity was comparatively even for each 60-minute period during a five-hour test period, and a common feature of both experiments is that the second hour showed the highest value. This holds true also throughout the placebo values in the study of the effect of drugs on the spontaneous activity (Table 8).

Diurnal activity, which was measured during a five-hour period, shows a considerably lower number of revolutions (Table 9). Also here the relative

deviation ($s_0\%$) is of quite another magnitude than that of the nocturnal activity. Especially the two last hours of each 60 minute period show high percentages for the standard deviations.

In order to get an idea of the importance of the administration of placebo, I have made a comparison test between untreated mice and mice which have been given placebo. The experiment was carried out as a cross-over test. Also here the second 60-minute period showed the greatest activity. However, it was not possible to demonstrate any significant difference in the results obtained from untreated mice and mice which had been given placebo during the first hour (Table 10).

Table 7. Mean aggregate values for nocturnal activity during a five-hour test period, when placebo was given over a period of fifteen days.

2 mice/cage	60 min.	120 min.	180 min.	240 min.	300 min.
Rev./cage	1 143 $\pm$ 23	2 565 $\pm$ 47	3 855 $\pm$ 63	4 998 $\pm$ 87	6 114 $\pm$ 126
$S_0\%$	7.9 $\pm$ 1.4	7.1 $\pm$ 1.3	6.3 $\pm$ 1.2	6.7 $\pm$ 1.2	8.0 $\pm$ 1.5
1 mouse/cage					
Rev./cage	835 $\pm$ 26	1 760 $\pm$ 43	2 599 $\pm$ 56	3 396 $\pm$ 69	4 116 $\pm$ 84
$S_0\%$	12.2 $\pm$ 2.2	9.5 $\pm$ 1.7	8.1 $\pm$ 1.5	7.9 $\pm$ 1.4	7.9 $\pm$ 1.4
Diff. $S_0\%$	4.3 $\pm$ 2.6 <i>df</i> 28 $P > 0.1$	2.4 $\pm$ 2.1 <i>df</i> 28 $P > 0.2$	1.8 $\pm$ 1.9 <i>df</i> 27 $P > 0.3$	1.2 $\pm$ 1.8 <i>df</i> 28 $P > 0.5$	0.1 $\pm$ 2.1 <i>df</i> 28 $P > 0.9$

Table 8. Mean values for nocturnal activity for each 60 minute period during a five-hour test period, when placebo was given over a period of fifteen days.

2 mice/cage	0—60 min.	60—120 min.	120—180 min.	180—240 min.	240—300 min.
Rev./cage	1 143 $\pm$ 23	1 422 $\pm$ 34	1 291 $\pm$ 25	1 143 $\pm$ 38	1 116 $\pm$ 56
$S_0\%$	7.9 $\pm$ 1.4	9.3 $\pm$ 1.7	7.5 $\pm$ 1.4	12.7 $\pm$ 2.3	19.3 $\pm$ 3.5
1 mouse/cage					
Rev./cage	835 $\pm$ 26	925 $\pm$ 24	838 $\pm$ 19	796 $\pm$ 24	721 $\pm$ 21
$S_0\%$	12.2 $\pm$ 2.2	10.5 $\pm$ 1.9	8.4 $\pm$ 1.6	11.3 $\pm$ 2.1	11.0 $\pm$ 2.0
Diff. $S_0\%$	4.3 $\pm$ 2.6 <i>df</i> 28 $P > 0.1$	1.2 $\pm$ 2.6 <i>df</i> 28 $P > 0.6$	0.9 $\pm$ 2.1 <i>df</i> 28 $P > 0.6$	1.4 $\pm$ 3.1 <i>df</i> 28 $P > 0.6$	8.3 $\pm$ 4.0 <i>df</i> 28 $P < 0.05$

Table 9a. Mean aggregate values for diurnal activity during a five-hour test period, when placebo was given over a period of fifteen days. One mouse per cage

	60 min.	120 min.	180 min.	240 min.	300 min.
Rev./cage	364 ± 31	616 ± 36	746 ± 43	802 ± 47	821 ± 50
S%	35.6 ± 5.8	25.6 ± 4.2	25.5 ± 4.0	26.2 ± 4.2	27.1 ± 4.3

Table 9b. Mean values for diurnal activity for each 60 minute period, when placebo was given over a period of fifteen days. One mouse per cage

	0—60 min.	60—120 min.	120—180 min.	180—240 min.	240—300 min.
Rev./cage	364 ± 31	252 ± 15	133 ± 13	56 ± 10	19 ± 8
S%	35.6 ± 5.8	26.1 ± 4.2	41.5 ± 6.8	80.1 ± 12.7	184.7 ± 29.2

Table 10. Variance analysis of 60 minute values (means) for mice which had and had not been administered saline

	df	Sum of squares	Mean square	Variance ratio	P
Between administration	1	12 675	12 675	1.61	> 0.05
Between days	5	17 358	3 472	2.27	> 0.05
Error	5	39 329	7 866	—	—
Total	11	69 362			

DISCUSSION

One must naturally take into consideration in experiments with mice the fact that they are nocturnal animals in contrast to human beings, particularly in studies of spontaneous activity.

The influence of factors such as daily variations, temperature, barometric pressure, hunger, age, contentment, and experience of the apparatus used on the spontaneous activity of rats has been demonstrated in earlier works (28, 30, 35, 38). In these experiments, where it has been primarily a question of attaining a relatively constant spontaneous activity from day to day, the difference in the spontaneous activity between diurnal and nocturnal

activity as well as the adaptiveness and experience of the apparatus have been of importance.

Male mice were used for this experiment as the ovarial cycle may have a certain effect (30).

The study of the effect of central depressant drugs on the spontaneous activity should be made preferably during the active period of the animals; thus, during nocturnal activity in the case of mice. This process has, in fact, been used by LJUNGBERG (37), SANDBERG (22) and others. As the test period for practical reasons should suitably coincide with the normal working hours of the laboratory, the experiments were carried out in a room where the light was switched off at 9 a.m. and switched on at 9 p.m. automatically. Obviously, a certain time was required for the mice to adapt themselves. The results show that it took ten days to attain a relatively even activity from day to day in the case of animals which not previously adapted their daily rhythm. According to LJUNGBERG (37) about a fortnight is necessary for the mice to adapt themselves and to learn to run around in the wheel, and the above experiments showed this to be sufficient.

However, where the animals had already had the chance of adapting their daily rhythm, five or six days would seem to be sufficient to attain an even activity from day to day (Figures 5 and 6). In the case where rats were used, the period necessary to attain an even activity is put at about a fortnight by JONES ET AL. (35). With the apparatus used in the present experiments the mice seem to get used to the arrangement more quickly and thus attain a relatively constant spontaneous activity. In the continuation of this experiment I have allowed the animals time to adapt their daily rhythm by always having some extra animals in the test room for a new series of experiments.

As can be seen from Table 2, the regression line for this experiment showed definite inclination. However, this inclination was so slight that it amounted to an increase of only $0.48\% \pm 0.24\%$. It was therefore probably of no significance in the assessment of the results for the subsequent experiments of the effect of drugs on the normal spontaneous activity. As the experiments also took the form of a cross-over test, this slight difference will be mostly eliminated.

It is worth mentioning that the mean values for the entire test and activity periods corresponded so well in both the experiments, with two animals per cage (Figures 4 and 5). In the regression calculations mean values of 10 526 and 10 700 respectively were obtained for the test period. The period between the two experiments was one year. In calculating the average

number of revolutions for the entire apparatus from day to day, the difference, as can be seen from the results, between the two experiments was even less ($10\,561 \pm 153$ and $10\,692 \pm 87$ respectively i.e. a difference of 1.25 %). Thus, the mice used showed a remarkably low variation from one experiment to the other when the experiments were carried out in the same conditions with the number of squirrel wheels used here.

The results also show the great difference between nocturnal and diurnal activity. RILEY & SPINKS (1) state that nocturnal activity shows greater variation than diurnal activity. Obviously, this would also be the case in these experiments if one compared the absolute values. However, this was of no importance for my experiments in which spontaneous activity was expressed as a percentage fraction of the control value for spontaneous activity (cf. Tables 7 and 8 with Table 9).

The *analyses of variance*, which are based on the placebo values over a five-day period, show that the variation *between* days was not statistically significant. This would seem to favour the use of only one placebo day in a five-day period in these experiments and thus administer test preparations in the other four days. A conventional cross-over test, as described above, in which the animals in half the total number of cases are given placebo and the other test preparations, is more likely to reduce the sources of error. Furthermore, the risk of cumulative intoxication is lessened as with this method the animals get the test preparations only every second day.

However, the variations *between* cages was significant (Tables 5, 6), i.e. each animal or pair of animals showed a comparatively even activity from day to day. Thus, for one animal or pair of animals the spontaneous activity could be high from day to day, for others it could be low, but it was always comparatively constant for each animal or pair of animals. This variation between individual animals has been observed in rats by SHIRLEY (38), who also has shown that the room temperature should be kept fairly constant as too great variations in temperature affect the spontaneous activity. However, this author says that a variation of between 21°C and 27°C would hardly seem to affect the activity.

The variation, which was noticed between the various cages in the present experiments, could obviously have been caused by the varying degrees of sluggishness of the wheels. In order to discover if this was the case, the mice, which showed a lively activity in one cage, were moved to cages where the mice showed low activity, and vice versa. However, the animals continued to show the same amount of activity, and therefore the sluggishness of the wheel probably played only a minor role. Furthermore, when new mice were brought in, wheels with greater sluggishness should

continue to show a low number of revolutions, but this has not been noticed. Moreover, a small difference in the sluggishness of different wheels probably plays no role, if the method with cross-over tests are to be used. In such a case, the animals use the same wheel both for placebo and when test preparations are administered.

The individual variations can be reduced by studying the animals beforehand and exchanging those animals which show great deviations from the mean value of spontaneous activity. Likewise animals which show great variations from day to day can be replaced by new animals. However, in these methodological experiments such a method has not been applied except where the animals had shown a marked decline in activity before the test started, i.e. during the week when they learned to use the wheels.

Tables 5 and 6 show values between 24 % and 34 % in a comparison based on the variation analyses between the relative deviations at the various reading times. The relative deviations may also give an idea of the number of cages required to attain the accuracy desired.

As the mean value of all the readings at each reading time was basis for the evaluation, the variation between the different days was that which was of the greatest interest. Should this variation be too great, the screening-test method described in Chapter I would be out of the question. The variation obtained in this experiment for a fifteen-day period was satisfactory both for one and two animals (Table 7).

The deviation as a percentage ($s_{\%}$) did not vary noticeably either for the aggregate values (Table 7) or for the one-hour values during the five-hour period (Table 8). This means that the test period could be extended over the entire five-hour period without affecting the accuracy to any great extent. On the other hand, DEWS (51) states that the photo-electric method, which is usually used during the diurnal period, has the smallest variation during the first fifteen-minute period, where it amounted to 15 % ($s_{\%}$) in his experiments. Both the period before and the period after had greater percentages of standard deviation. When assessing the results reported by BROWN ETAL (14) one finds that even these have a high percentage of deviation, when the experiments extended over a long period and where the test arrangements included the use of the photo-electric method.

One found here also in a comparison with diurnal activity (Table 9) that the percentage of standard deviation for a five hour period was smallest during the first two hours but considerably higher than for nocturnal activity. The large deviation of diurnal activity also speaks in favour of testing during nocturnal activity drugs which are expected to cause reduced

spontaneous activity. It was quite a different matter for stimulating drugs, as these can produce activities many times greater than the normal.

Table 8 shows that the activity was relatively constant throughout the entire five hour period. SZYMANSKI (17) in his studies demonstrates 16 periods of rest and 16 periods of activity in twenty-four hours; others (14, 25) as well as DEWS (51) indicate a high initial spontaneous activity. Probably to a large extent this can be attributed to the "burst" which the animals get when they are placed in new surroundings. Also the injection can play a certain role in bringing about a high initial period of activity (25).

In an experiment of this kind it is obviously an advantage if the test period can be extended over a longer period of time, as this makes it possible to discover the effect of drugs having long induction time, such as reserpine (cf. Chapter III).

The percentage deviation for aggregate values was greatest during the first hour and in all the cases, with the exception of the fifth hour, the values for one mouse were somewhat higher than for two mice. However, in no case was there any definite statistical difference.

As can be seen from the results, the relative standard deviation for one animal per cage has also been calculated for the first 30 minutes, as in this case the time needed for the administration probably plays a less important role in the results than in the case of two animals per cage, where the difference in time between the first and the last administrations can be as much as 15 minutes.

Here the relative standard deviation was 18 %, i.e. approx. 1.5 times greater than the calculated standard deviations for the 60-minute periods with the exception of the last hour with two mice per cage. These results thus show a tendency opposite to that reported by DEWS (51). No difference could be established with certainty in a comparison study of the spontaneous activity of mice, which were either treated or not treated with placebo. This must indicate that the normal spontaneous activity of the animals was not upset by the administration, as the difference between untreated mice and animals which had been given placebo was insignificant (Table 10). This would mean, that control values could be used for untreated mice. On the other hand, SEARLE & BROWN (33) in an experiment involving the subcutan administration of amphetamine sulphate with white rats have noted a marked effect together with increased spontaneous activity. However, in these experiments placebo has always been given in order to eliminate the possible effect of the injection on the spontaneous activity.

From the above it can be seen that the apparatus used with the method here has such an accuracy that it appears to be well suited for a screening-

test of drugs in the manner already described. Here there is probably no difference whether there are one or two animals in the cage. It is true that with two animals there is generally a somewhat slighter variation. However, on the other hand, having one animal per cage results in a saving of time in the administration and also of costs. Further test of known drugs could determine the suitability of the method as well as whether there should be one or two animals per cage.

As has been pointed out earlier, studies of the sedative effect should be carried out during the active period of the animals. The large deviation, which occurred during diurnal activity, would also exclude the study of sedative effect during this period.

CHAPTER III

SOME DRUGS INFLUENCING THE NORMAL SPONTANEOUS ACTIVITY

AS was mentioned in the introduction, spontaneous activity is considered to be a suitable method if it is desired to study the sedative effect of a substance. However, in order to be able to form an appreciation of the effect of various substances on the spontaneous activity of animals, a practical first step is to determine the normal variation in their spontaneous activity when an inactive substance is administered. In the previous chapter it was described how a physiological saline solution was used to determine the normal variation. This method was found to give a satisfactorily low variation over a five hour test period with both one and two animals per cage, in respect of both aggregate values and values for each period.

In order to judge whether the method can be used to determine the effects of various substances on spontaneous activity it is also necessary that the administration should produce an effect differing from the norm. LJUNGBERG (37) in an earlier study has demonstrated that a linear dose-effect curve exists for phenobarbital sodium when this is administered subcutaneously to mice, but the question of whether other substances affecting the central nervous system also produce this effect has not been studied in detail.

In a screening test the principal consideration is to select substances which may possibly be of interest and above all not to lose any active substance. About thirty substances known to affect the central nervous system were therefore studied in the present chapter with reference to their effect on spontaneous activity. This activity was compared with that of animals treated with placebo in a cross-over test already described in Chapter I. Aggregate values for the spontaneous activity of the test animals during a five hour period were recorded at certain intervals and expressed as a percentage of the corresponding activity on placebo. The results for the various substances were plotted on graphs with the spontaneous activity in percentage terms on the ordinate and the time on the abscissa. To obtain a comparison with the previously determined standard deviation for physiological saline, twice the standard deviation was plotted on the graphs. Thus in theory at any given point about 95 % of the placebo values should lie within these limits. If, on the other hand, the deviation is counted in

one direction only, not more than 2.5 % will lie outside this limit. If instead three times the standard deviation is selected as a limit, 99.7 % of the placebo values determined as mean values from day to day will lie within these limits. If aggregate values are selected for assessment of the results, read-off values are obtained direct. This also provides some indication of a suitable time at which to take readings for the various types of preparation, if it is desired to take only one reading. The method in this case is also the simplest possible one. If it is desired to compare various periods of time during the five hour period with each other, they can be calculated easily; this may be the case if the substance has proved to be of interest.

Each individual value for each cage was noted. This made it possible, if desired, to establish by a *t*-analysis the difference in spontaneous activity either as compared with placebo or for the purpose of making comparison between different substances.

Noting each individual value for each cage also made it possible to find out whether any mechanical or other defect had arisen in the course of the test. These aberrant values had not been taken into consideration in the assessment of the results, and as a rule the corresponding comparison values had also been excluded, even though the averages had been used in the assessment. If only one reading had been available for the entire apparatus, such a procedure would not have been possible.

To prevent influence of acute toxic effects the doses administered were selected as to fall safely short of LD₅₀. The latter was determined as already described in Chapter I. However, where LD₅₀ was above 1 000 mg/kg b.w., the medium lethal dose was not determined, since so low toxicity can be assumed to be insignificant for the assessment of spontaneous activity with the doses used.

Previous pharmacological studies of substances also gave some guidance as to the selection of the dose. In some cases, however, it proved necessary to make preliminary tests in order to arrive at a suitable dosage.

If the hypnotic dose (HD₅₀) was considered to be important to the investigation and to the assessment of the dose levels used, this value was also determined as described in Chapter I.

Since potentiated narcosis is among the foremost and simplest methods for screening substances with a presumed central effect, I also considered it of interest to compare this method with a dose level showing an effect on spontaneous activity.

The following drugs have been studied in the course of the investigation:

Hypnotic and Sedative Drugs*Aldehydes:*

Chloral hydrate

Alcohols:

Ethylalcohol

Methylpentynol

Ureids:

Bromural

Carbromal

Ectylurea

Phenylcrotonylurea

Barbiturates:

Hexobarbital sodium

Amobarbital

Phenobarbital sodium

Glutarimides:

Glutethimide

Thalidomide

Tranquilizers

Reserpine

Chlorpromazine

Haloperidol

Meproamate

Emylcamate

Antidepressant Drugs

Imipramine

Iproniazid

Antihistamines

Promethazine

Phenbenzamine

Analgesic and Antipyretic Drugs*Strong analgesic:*

Morphine

Non-narcotic analgesics:

Acetylsalicylic acid

Phenazone

Central Stimulants

Amphetamine

Caffein

Diethylpropione

Hypnotic and Sedative Drugs*Aldehydes:*

Chloral hydrate was tested as a representative of the aldehyde group. It is the oldest, synthesized pharmaceutical belonging to the sedative and hypnotic group of drugs having been introduced in therapy in 1869, and is still one of our more commonly used hypnotics.

Chloral hydrateLD₅₀: 1 099 (1 012—1 167) mg/kg b.w.HD₅₀: 539 (490— 634) mg/kg b.w.

Doses: 2 mice per cage: 200 and 400 mg/kg b.w.

1 mouse per cage: 200 and 300 mg/kg b.w.

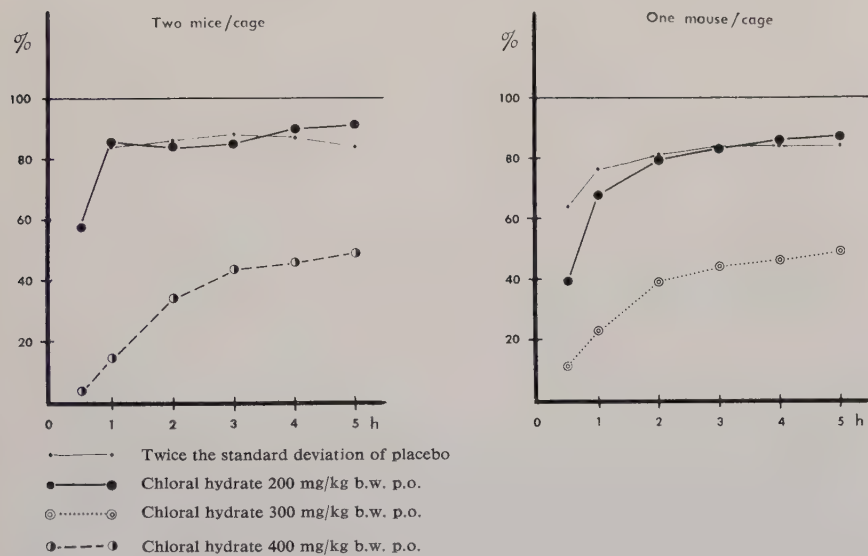
Potentiated narcosis 200 mg/kg b.w.: 68.2 ± 2.8 min.Control: 32.5 ± 3.2 min.*df.* 18 $P < 0.001$ 

Fig. 7. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The greatest reduction in spontaneous activity with a dosage of 200 mg/kg b.w. was obtained for one animal per cage (Figure 7). Thirty-minute values have also been included in the graphs, as these showed the greatest reduction in both cases. A single animal in a cage showed here a decrease to 39 % of placebo, while two animals gave a decrease to 57 %. DEGERHOLM ET AL. (52) give ED_{50} for chloral hydrate determined by the climbing test according to KNEIP (12) at 30 minutes after administration as 166 mg/kg b.w. Already after one hour the reduction was considerably less in both cases, and for two animals per cage the reduction in spontaneous activity was less than twice the standard deviation (Figure 7).

The dose of 400 mg/kg b.w. was administered only to two mice per cage and produced a sharp reduction in spontaneous activity especially during the first 30 and 60 minutes. The administered dose was well below LD_{50} but was relatively high in relation to HD_{50} .

The dose of 300 mg/kg b.w. produced a reduction in spontaneous activity for one animal per cage which was roughly comparable to that obtained by 400 mg/kg b.w. with two animals per cage.

Since the value of 68 % for the first hour with one animal per cage may be near the limit of what can be established by a *t*-analysis, such an analysis was performed here. It was found that chloral hydrate in this dosage produced an effect on spontaneous activity which showed a significant deviation from placebo ($t_d = 3.22$ *df.* 18 $P < 0.001$).

The values for the various dosages indicate that a dose-effect relationship may exist for chloral hydrate (see Chapter IV).

A relatively small increase in the dose evidently lead to a great increase in the effect on spontaneous activity. This may point to a narrow "sedative range", which is also supported by the circumstance that the hypnotic dose is close to the doses which produced an effect on spontaneous activity.

The values obtained for toxicity and hypnotic effect show fairly good agreement with those found by REINHARD ET AL. (53), DEGERHOLM ET AL. (52) and RICE & MCCOLL (54).

Alcohols:

Ethylalcohol and methylpentynol were tested as typical alcohol preparations — ethylalcohol mainly because it often gives a synergetic effect with sedative drugs and has therefore also been the subject of many pharmacological studies of combined action [cf. EEROLA (55), SANDBERG (56) and others (57, 58)].

Methylpentynol was first studied by MARGOLIN ET AL (59) since then both the alcohol and its carbamate have been the subject of pharmacological studies by BARNES ET AL (60), MARLEY (61) and others. Methylpentynol has also been used as a control substance for the testing of new substances with a sedative and hypnotic effect (21, 53, 62, 63).

Ethylalcohol

 C_2H_5OH

LD_{50} : about 7.5 g/kg b.w. in 30 % w/v solution

Doses: 2 mice per cage: 3 g/kg b.w. in 12 % w/v solution

1 mouse per cage: 3 g/kg b.w. in 12 % w/v solution and 1.5 g/kg b.w. in 6 % w/v solution.

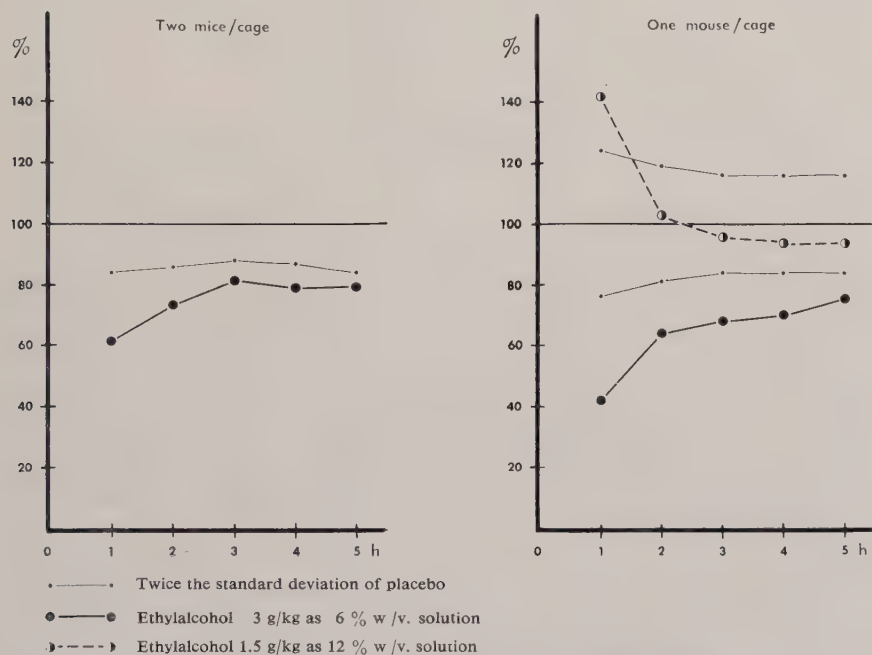
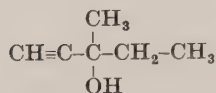


Fig. 8. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The dose of 3 g/kg b.w. must be regarded as relatively high in relation to LD_{50} and the animals were also observed to be noticeably affected after administration. The greatest reduction was obtained during the first hour and was most marked with one animal per cage. The smaller dosage was given only to animals who were alone in a cage and here stimulation was observable during the first 30 and 60 minutes.

STEWART (28) has studied the effect of various strengths of alcohol on spontaneous activity in white rats using the squirrel-wheel method. Alcohol was given in the food and no change in spontaneous activity was observed up to 20 %, while higher percentages resulted in a decrease. The number of experimental animals, however, was small.

Methylpentynol



LD₅₀: 744 (688—808) mg/kg b.w.

HD₅₀: 315 (299—332) mg/kg b.w.

Doses: 2 mice per cage: 200 and 300 mg/kg b.w.

1 mouse per cage: 200 and 300 mg/kg b.w.

Potentiated narcosis 200 mg/kg b.w. > 405 min.

Control: 32.5 ± 3.2 min.

df. 18 $P < 0.001$

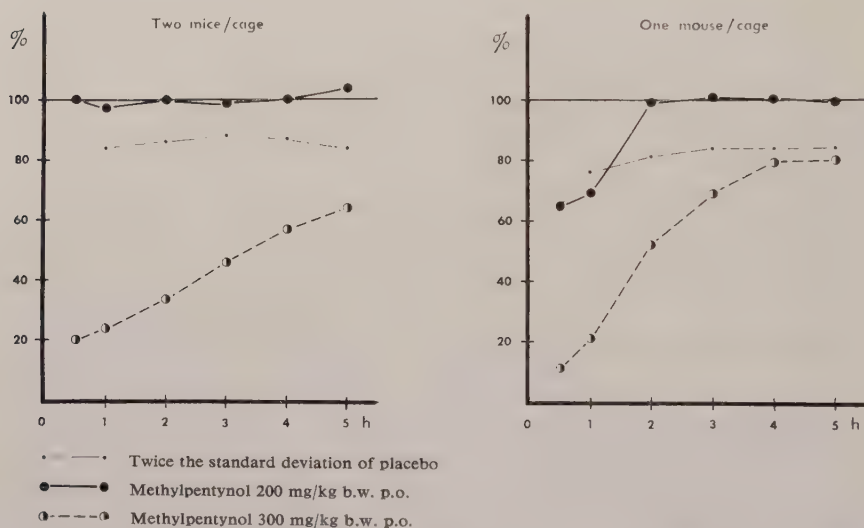


Fig. 9. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

In the case of two mice per cage, no reduction in spontaneous activity was obtained at 200 mg/kg b.w., while an increase to 300 mg/kg b.w. reduced spontaneous activity to only 24 % of that with placebo during the first hour and down to as low as 20 % during the first 30 minutes. The latter dose is also very close to the hypnotic dose, i.e. the preparation showed a very narrow "sedative range" in these studies.

GROSS ET AL. (62) also obtained the effect after 30 minutes and a short duration when they determined the effect with rotating rod. REINHARD ET AL. (53) found the toxicity to be 940 mg/kg b.w. and HD₅₀ at 360 mg/kg

Ureids:

In view of the interesting pharmacological results obtained with the two geometrical isomers of ectylurea by FANCHER & LIM (71) and LJUNGBERG & STRÖM (70). It would be of interest to test two similar geometrical isomers which have not previously been subjected to pharmacological study; in these the ethyl radical was replaced by phenyl. (These substances were synthesized and kindly made available by Dr. C.-G Lindblad of the Swedish Pharmacies' Central Laboratory).

$$\begin{array}{ccccccc} \text{H}_3\text{C} & & & & & & \\ & \diagdown & & & & & \\ & \text{CH} & - & \text{CH} & - & \text{C} & - & \text{NH} & - & \text{C} & - & \text{NH}_2 \\ & \diagup & & | & & || & & & & || \\ \text{H}_3\text{C} & & & \text{Br} & & \text{O} & & & & \text{O} \end{array}$$
 $df. 17 \ P < 0.001$

A clear reduction of the spontaneous activity was obtained with both one and two animals per cage. The reduction was however greater for one animal than for two, as in the latter case 200 mg/kg b.w. did not give as much reduction as 100 mg/kg b.w. for one animal per cage. As the figures show, the effect persisted throughout the test period.

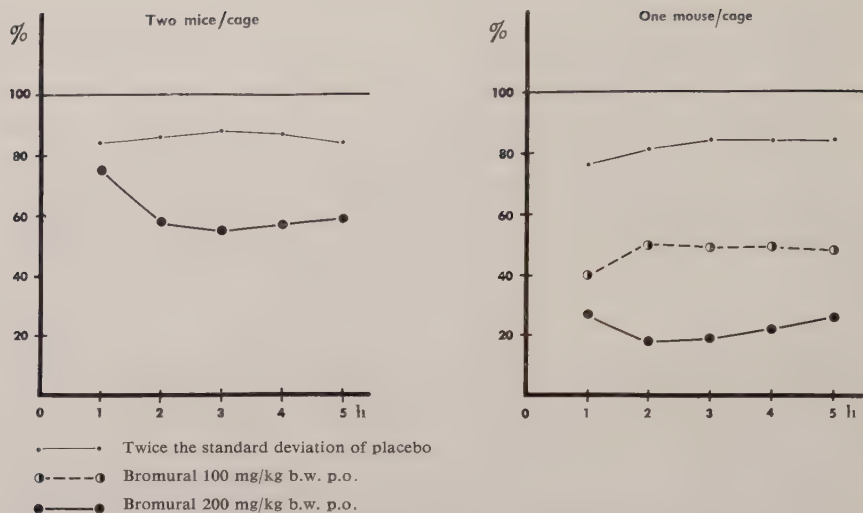
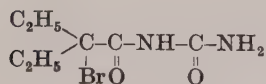


Fig. 10. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

According to v.d. EECKHOUT (64), 200 mg/kg b.w. had a hypnotic effect on rabbits and dogs. The results on different animals are however described as greatly variable, and since the test material used was also very small, no comparison can be made with the results obtained here.

Carbromal



LD₅₀: 1 165 (1 078—1 398) mg/kg b.w.

Doses: 2 mice per cage 200 mg/kg b.w.

1 mouse per cage 200 mg/kg b.w.

Potentiated narcosis 200 mg/kg b.w.: $173,6 \pm 23,7$ min.

Control: $41,9 \pm 3,8$ min.

df. 17 $P < 0.001$

As in the previous tests with bromural, a greater reduction in spontaneous activity was obtained here with one mouse per cage. The reduction was, as the results show, less than with the same dosage of the previous ureid. The *t*-analysis showed that the effect was significant during the first hour in the case of one animal per cage ($t_d = 5.10$ *df.* 58 $P < 0.001$).

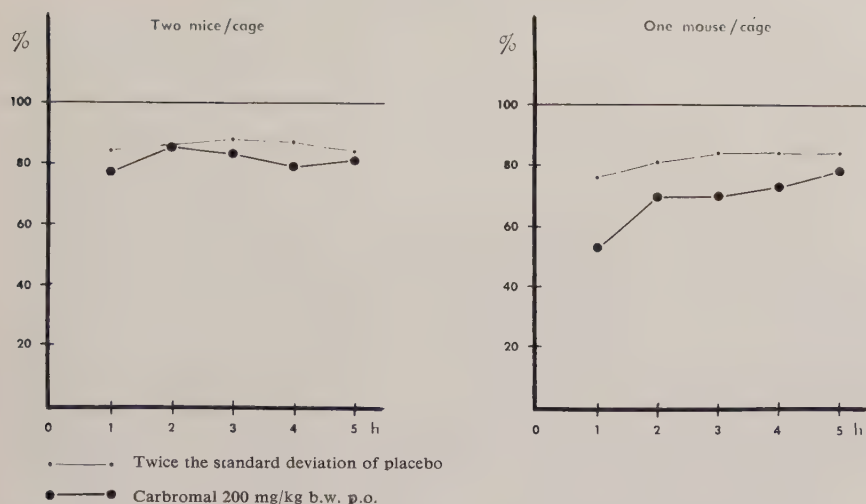
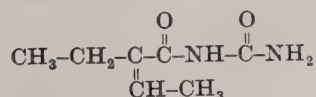


Fig. 11. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

Ectylurea



$\text{LD}_{50} > 1\,000 \text{ mg/kg b.w.}$

Doses: 2 mice per cage: 200 mg/kg b.w.

1 mouse per cage: 100 mg/kg b.w.

Potentiated narcosis 200 mg/kg b.w.: 40.6 ± 3.6

Control: 30.6 ± 1.5

$df. 17 P < 0.001$

A sharp reduction in spontaneous activity was observable here whether one or two animals per cage were used. In both tests the reduction was of about the same magnitude, although only half the dose was administered to one mouse per cage.

Since the hypnotic dose is very close to the toxic dose, which according to LJUNGBERG & STRÖM (70) is above 4.5 g/kg b.w. when using oral administration to mice, the "sedative range" of the substance seems to be great. This has also been noted with rats, where the substance produced sedation in doses of 1 % of LD_{50} (69).

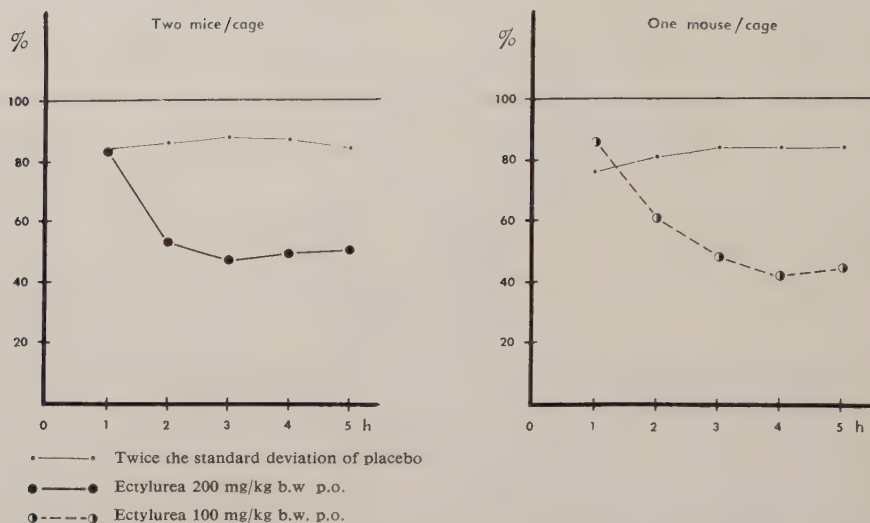


Fig. 12. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

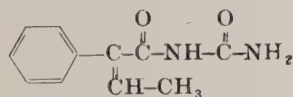
As has been emphasized in papers by FANCHER & LIM (71) and LJUNGBERG & STRÖM (70), the low-melting geometrical isomer does not by any means show the same pharmacological properties as the high-melting isomer tested here. This will be dealt with in more detail in Chapter V.

WEAVER & MIYA (74) have demonstrated that ectylurea in a dosage of 50 mg/kg b.w. administered intraperitoneally to mice did not have any effect on their co-ordination as determined with rotating rods, but that the same dosage lowered the spontaneous activity determined by the photo-electric method to 50%. KINNARD & CARR (11) have obtained similar results using the same method of investigation.

Since ectylurea is a relatively new substance compared to the ureides previously studied, bromural and carbromal, it would be of interest to compare these two substances with ectylurea in a cross-over test at the same level of dosage. The tests were carried out with one animal per cage at a dose of 100 mg/kg b.w. Ectylurea in a cross-over test against bromural gave during the second hour a greater effect ($t_d = 4.35$ $df. = 44$ $P < 0.001$).

The test with carbromal — also at a dose of 100 mg/kg b.w. — gave likewise a significant difference in favour of ectylurea ($t_d = 6.72$ $df. = 46$ $P < 0.001$).

Phenylcrotonylurea



high-melting isomer

 $\text{LD}_{50} > 1\,000 \text{ mg/kg b.w.}$

Doses: 1 mouse per cage: 50, 100 and 200 mg/kg b.w.

Potentiated narcosis 200 mg/kg b.w.: $87.8 \pm 7.7 \text{ min.}$ Control: $42.8 \pm 3.2 \text{ min.}$ $df. 18 < 0.001$

low-melting isomer

 $\text{LD}_{50} > 1\,000 \text{ mg/kg b.w.}$

Dose: 1 mouse per cage 100 mg/kg b.w.

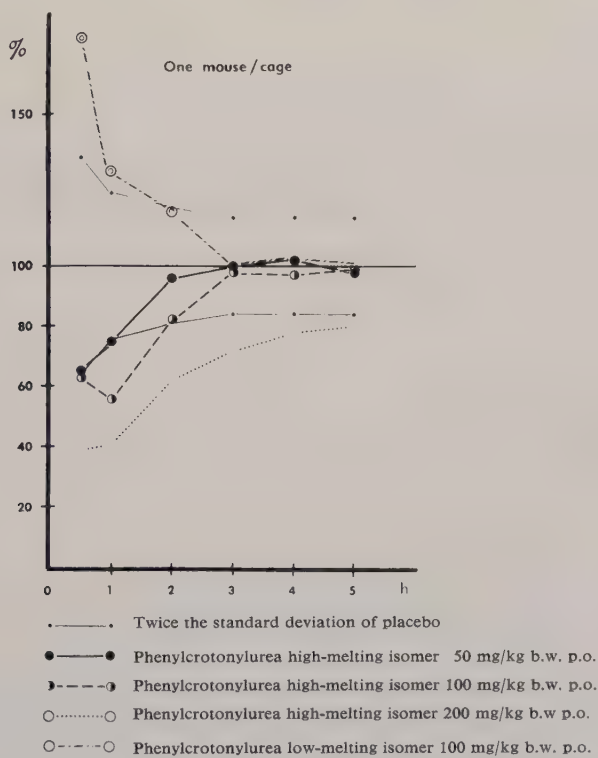
Potentiated narcosis 200 mg/kg b.w.: $40.0 \pm 3.8 \text{ min.}$ Control: $42.8 \pm 3.2 \text{ min.}$ $df. 18 P > 0.05$ 

Fig. 13. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The high-melting isomer gave a maximum reduction of spontaneous activity of 41 %, 56 % and 76 % respectively during the first hour, while with the low-melting isomer an increased activity of about 175% of placebo could be noted during the first 30-minute period. Thus the same interesting results as those with ectylurea were obtained (69, 70).

With the potentiated narcosis only the high-melting isomer gave a definite effect.

Barbiturates :

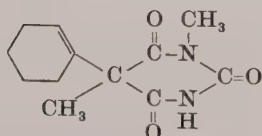
The barbiturates are undoubtedly the most closely studied and from a clinical point of view the most commonly used chemical group within hypnotics and sedatives. Fischer's synthesis in 1903 of barbitol encouraged to the synthesizing and testing of thousands of barbiturates in which the connection of chemical constitution and biological effect had been systematically studied. Here an investigation has been made of one preparation with short, one with medium and one with long duration, namely hexobarbital, amobarbital and phenobarbital.

In 1932 WEESE and SCHARPF (75) described hexobarbital both pharmacologically and clinically. As has been pointed out earlier, the substance is very useful in experimental studies of potentiated narcosis on animals (2).

Amobarbital was found to be the most active in a series of barbituric acid derivatives, synthesized by SHONLE & MOMENT (76). Its pharmacodynamic properties compared with those of other barbiturates have later been studied by SWANSON & PAGE (77) as well as by DOX & HJORT (78) and others.

Phenobarbital is the second oldest of the clinically used barbiturates. It was introduced in 1912 when IMPENS (79) dealt with its pharmacology. The same year LOEWE (80) and JULIUSBURGER (81) tried the substance clinically. Since then phenobarbital has been used in several investigations, as this substance very often has been applied as a comparative substance in research of newer substances with assumed sedative or hypnotic effect.

Hexobarbital sodium



Hexobarbital

LD₅₀: 372 (346—401) mg/kg b.w.

HD₅₀: 67 (60— 76) mg/kg b.w.

Doses: 2 mice per cage: 50 and 75 mg/kg b.w.

1 mouse per cage: 50 and 75 mg/kg b.w.

Both of the administered doses were relatively high compared with HD₅₀, but yet fell safely short of LD₅₀. Despite the high doses the reduction of the spontaneous activity for two mice per cage was moderate.

REINHARD ET AL (53) found the LD₅₀ for hexobarbital to be 468 mg/kg

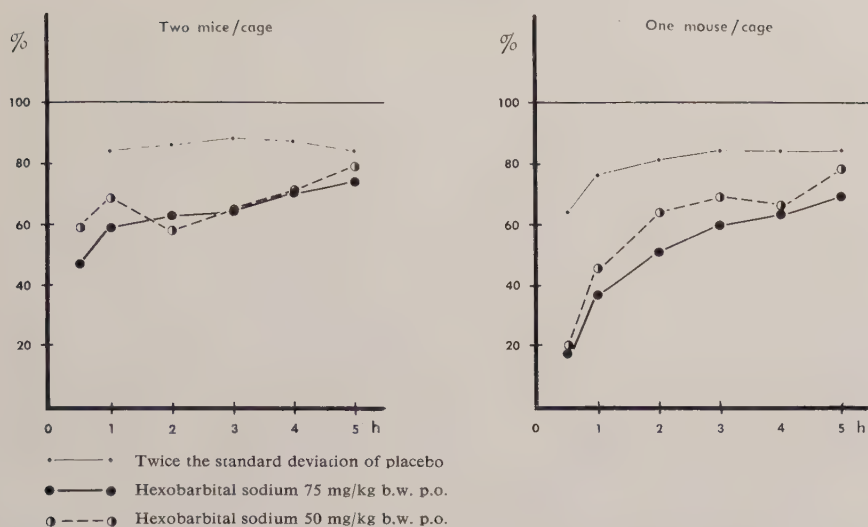
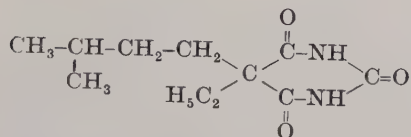


Fig. 14. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

b.w. after oral administration to mice, and HD_{50} to be 130 mg/kg b.w. CAVIEZEL & BAILLOD (82) have studied hexobarbital using an apparatus, designed in such a way that it works as a jiggle cage, in which the entire activity is registered, and at the same time as a revolving drum. However, the latter contrary to this apparatus and most others, has a vertical shaft. In this case the "running activity" proved to be significantly lowered after oral administration of 120 mg/kg b.w., but the entire activity did not show any definite change.

Amobarbital



LD_{50} : 828 mg/kg b.w. (582—1 008) mg/kg b.w.

HD_{50} : 124 mg/kg b.w. (119—130) mg/kg b.w.

Doses: 2 mice per cage: 50 and 100 mg/kg b.w.

1 mouse per cage: 50 and 100 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: 78.8 ± 7.6 min.

Control: 47.2 ± 3.0 min.

$df. 18$ $P < 0.001$

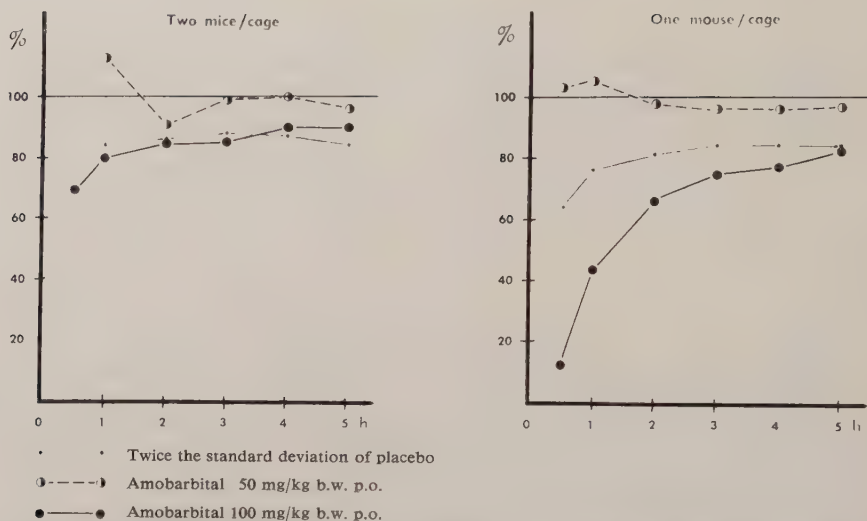
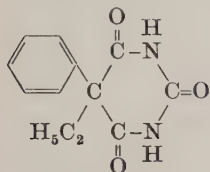


Fig. 15. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The lower dose showed no definite effect. Only when the doses were increased to 100 mg/kg b.w. effects were obtained for both one and two animals per cage. Despite the fact that the dose is close to HD_{50} for two animals per cage a reduction of only 70 % of the placebo effect was obtained during the first 30 minutes. After that the effect was fairly constant during the rest of the test period. However, with one animal per cage, a strong reduction of spontaneous activity with a rather transient effect was obtained. In computing the t -analysis of the first hour a significant deviation from placebo was obtained ($t_d = 2.33$ $df. = 68$ $P < 0.05$).

KINNARD & CARR (11) have studied amobarbital sodium using the photo-electric method and rotating rod. The photo-electric method gave an increased activity with the i. p. doses 20, 40 and 60 mg/kg b.w., while the doses 75 and 80 mg/kg b.w. did not produce any change, and 90 mg/kg b.w. a strong reduction of the activity. Rotating rods gave a strong reduction of the co-ordination in doses 60 and 70 mg/kg b.w., while 20 and 40 mg/kg b.w. did not produce any effect; 80 and 90 mg/kg b.w. have not been tested. In oral administration REINHARD ET AL. (53) have determined HD_{50} at 129 mg/kg b.w. and LD_{50} at 560 mg/kg b.w. in mice.

Phenobarbital sodium

*Phenobarbital*LD₅₀: 248 (229—269) mg/kg b.w.HD₅₀: 119 (114—124) mg/kg b.w.

Doses: 2 mice per cage: 50 mg/kg b.w.

1 mouse per cage: 50 mg/kg b.w.

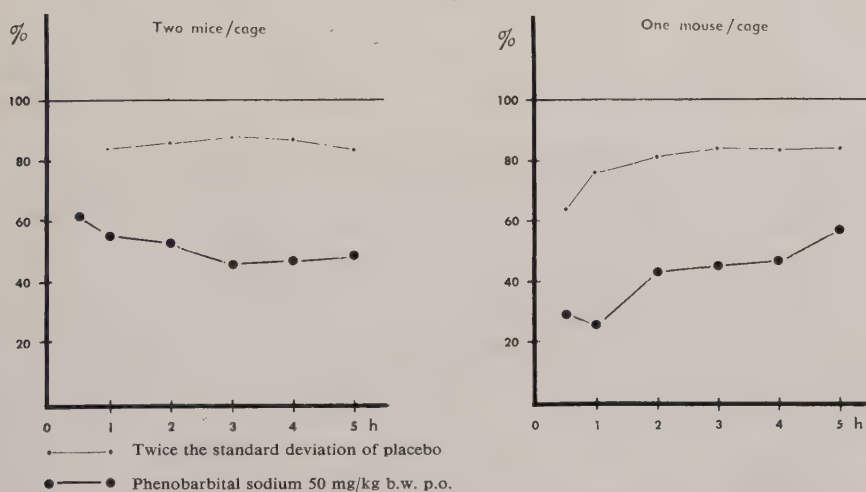
Potentiated narcosis 50 mg/kg b.w.: 87.8 ± 10.1 min.Control: 47.2 ± 3.0 min.*df.* 18 $P < 0.001$ 

Fig. 16. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

LJUNGBERG (37), in his study of phenobarbital sodium, has used doses between 9.5—80 mg/kg b.w. subcutaneously. A dose of 50 mg/kg b.w. orally has thus been considered suitable. Both with one and two animals the effects obtained were considerably greater than twice the standard deviation.

As can be seen, the effects in both one and two animals per cage were almost alike, with the exception that with one animal per cage a greater reduction of the spontaneous activity was obtained during the 30-minute and 60-minute periods.

TRIPOD & GROSS (9) in their studies with rotating rod have demonstrated that an effect can be obtained at an interval between oral doses of 25—70 mg/kg b.w., and with the same method JANSEN ET AL (83) gives ED_{50} as 80 mg/kg b.w. subcutaneously for phenobarbital. GROSS ET AL. (62) give the toxicity as 250 mg/kg b.w., KUNZ ET AL. (21) as 300 mg/kg b.w., REINHARD ET AL. (53) as 325 mg/kg b.w., SCHALLEK ET AL. (15) as 240 mg/kg b.w. — all with oral administration of phenobarbital in mice. REINHARD ET AL. (53) give the median hypnotic dose (HD_{50}) as 89 mg/kg b.w. orally to mice, while LJUNGBERG (37) in subcutaneous administration to mice gives the dose as 149 mg/kg b.w. and the lethal dose (LD_{50}) when using the same method of administration as 255 mg/kg b.w.

Glutarimides:

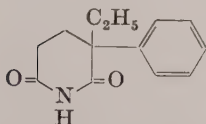
The search for new drugs with sedative and hypnotic effects resulted in a new chemical group within sedatives and hypnotics, *i.e.* glutarimides. Of these, glutethimide and thalidomide have been studied here.

Glutethimide was synthesized by TAGMAN ET AL. (84), and animal experiments have later on been made by GROSS ET AL. in 1955 (62), who claimed that the duration of glutethimide is similar to that of methylpentynol, but more potent.

The same year MÜLLER & ROHRER (85) published clinical investigations on the substance.

Thalidomide was synthesized and tested on animals by KUNZ ET AL. (21), who compared the substance with phenobarbital, methylpentynol, glutethimide, etc. However, the substance has shown marked teratogenic properties, and it is therefore no longer used in therapy.

Glutethimide



LD_{50} : 485 (411—529) mg/kg b.w.

HD_{50} : 173 (157—192) mg/kg b.w.

Doses: 2 mice per cage: 100 and 200 mg/kg b.w.

1 mouse per cage: 100 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: 332.1 ± 25.7 min.

Control: 32.5 ± 3.2 min.

df. 18 $P < 0.001$

The greatest effect was obtained during the first half-hour with both one and two animals per cage. As was the case in the investigation of the previous preparations the largest reduction was obtained with only one animal

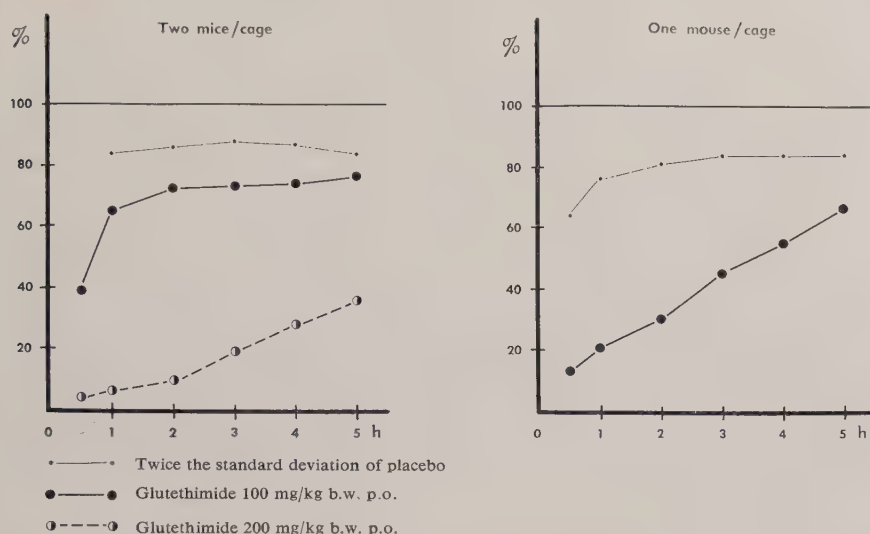
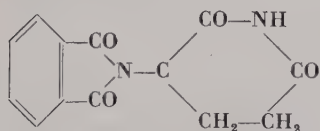


Fig. 17. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

per cage. 200 mg/kg b.w. which is higher than HD_{50} gave a reduction of spontaneous activity down to 4 % of that of placebo. *TRIPOD & GROSS* (9) have demonstrated a dose-effect relation within the range 15—100 mg/kg b.w. orally with rotating rods, and as pointed out earlier, *KUNZ ET AL.* (21) state that 75 mg/kg b.w. orally reduced spontaneous activity to at least the half when using a variant of jiggle cage.

GROSS ET AL. (62) have also studied the influence of the substance on the co-ordination with rotating rod and a maximum effect with doses between 50—100 mg/kg b.w. was obtained after 30 minutes. *GROSS ET AL.* (62) give the toxicity as 500 mg/kg b.w., and *KUNZ ET AL.* (21) as 600 mg/kg b.w. when given orally to mice, which well corresponds with the value obtained here.

Thalidomide



$LD_{50} > 1\ 000$ mg/kg b.w.

Doses: 2 mice per cage: 200 and 400 mg/kg b.w.

1 mouse per cage: 100 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: 88.0 ± 8.3 min.

Control: 62.2 ± 5.8 min.

$df.18$ $P < 0.05$

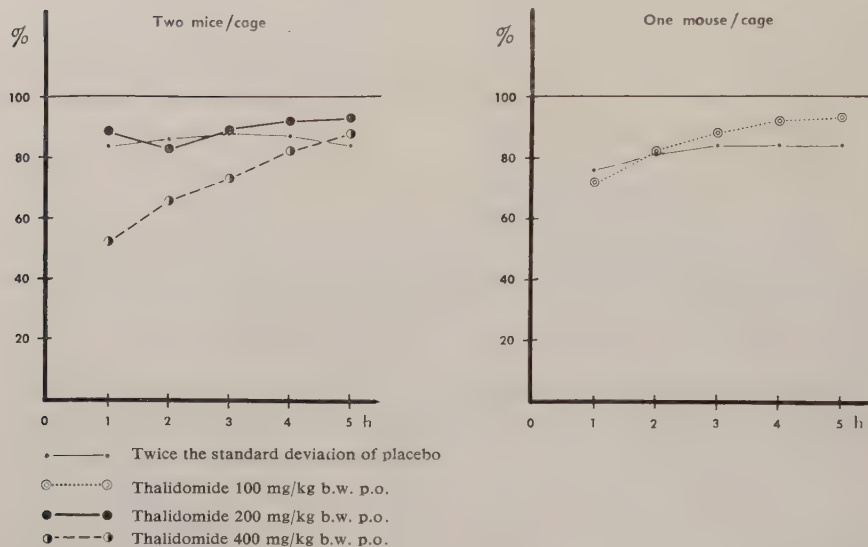


Fig. 18. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

With one animal per cage given 100 mg/kg b.w. the effect was equally great as with 200 mg/kg b.w. and two animals per cage. The dose 400 mg/kg b.w. gave an increased effect, which reached its maximum during the first hour. Since, according to KUNZ ET AL. (21), the hypnotic dose of the preparation is more than 5 g/kg b.w. orally in mice, this substance seems to have a large "sedative range". In the study of spontaneous activity with the jiggle cage method, KUNZ ET AL. (21) state that 100 mg/kg b.w. orally reduce the spontaneous activity to at least half of the normal one.

In a study of β -substituted glutarimides I also tested unsubstituted glutarimide (86), but it failed to give any reduction of the spontaneous activity in the corresponding dose (100 mg/kg b.w.).

Tranquilizers:

In the present investigation reserpine, chlorpromazine, haloperidol, meprobamate and emylcamate have been studied.

The sedative effect of reserpine has been demonstrated in animal experiments by BEIN ET AL. (87), PLUMMER ET AL. (88) and others. A number of animal experiments and clinical studies have subsequently been carried out.

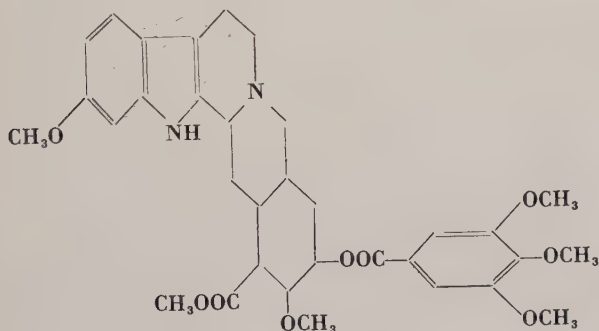
Chlorpromazine, which chemically belongs to the phenothiazine derivatives, was studied on animals by COURVOISIER ET AL. (89) and has later, as reserpine, been the subject of several experimental investigations.

JANSEN ET AL. (83) were the first ones to synthesize and pharmacologically study haloperidol, which is alleged to have the same qualitative, but larger quantitative effect than chlorpromazine.

Meprobamate belongs chemically to the propandiol derivatives, and like mephensine has a central muscle relaxant effect. The fundamental pharmacological studies in animals have been carried out by BERGER (16).

Emylcamate has been studied by MELANDER (27) who in his experiments compared the substance with meprobamate and found it to be superior to the latter in reducing spontaneous activity, estimated by the photo-electric method.

Reserpine



$LD_{50} > 1\ 000\ \text{mg/kg b.w.}$

Doses: 2 mice per cage 10 and 50 mg/kg b.w.

1 mouse per cage 10, 20 and 50 mg/kg b.w.

Potentiated narcosis 10 mg/kg b.w.: $57.8 \pm 3.9\ \text{min.}$

Control: $62.2 \pm 5.8\ \text{min.}$

$df. 18\ P > 0.05$

(Hexobarbital sodium i.p.: 120 minutes after administration of reserpine).

A reduction of spontaneous activity was noted with both one and two animals per cage. The continuous reduction of spontaneous activity was of great interest. This can be clearly seen in figure 20, which gives a comparison between the preparation and placebo for each 60-minute period. Even the high dose (50 mg/kg b.w.) showed a relatively small reduction during the first hour which later increased gradually. With one animal

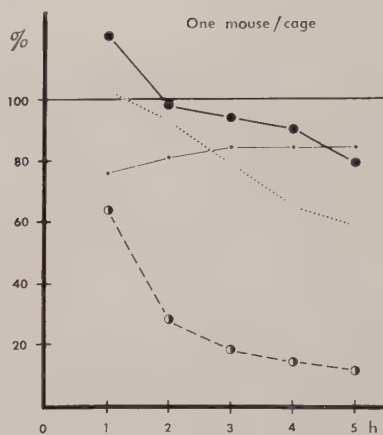
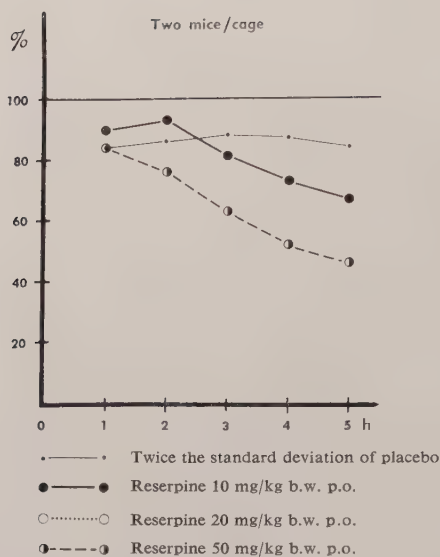


Fig. 19. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

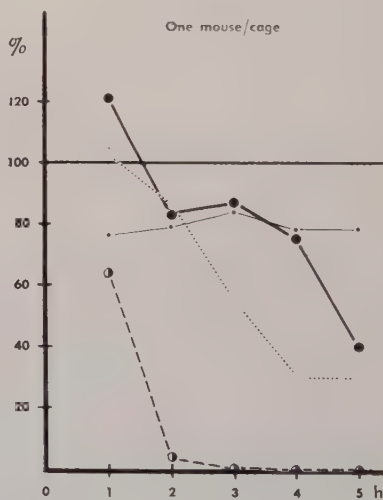
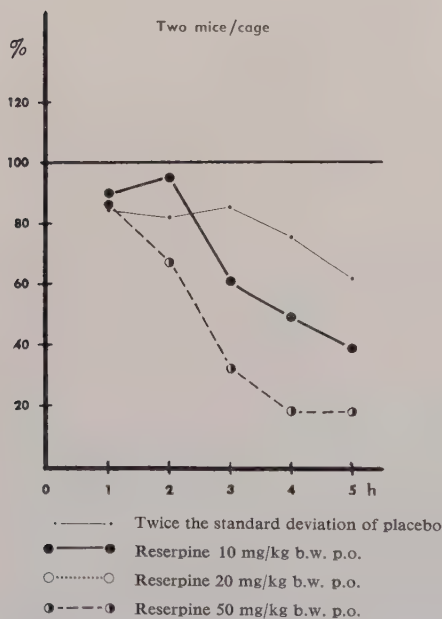


Fig. 20. Spontaneous activity in % of placebo for each 60-minute period during a five hour test period.

per cage the activity during the first 60-minute period was 64 %, which during the next 60-minute period fell to 4 %. With the doses 10 mg/kg b.w. and 20 mg/kg b.w. no reduction of spontaneous activity was obtained during the first hour, but during the subsequent 60-minute period spontaneous activity had fallen to 80 % of that of placebo. Thus the gradual reduction of spontaneous activity during the test period is common for one as well as two animals per cage and also for low and high doses.

The results obtained indicate that a dose-effect relation may exist.

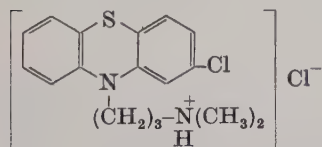
Since the maximum effect was reached during the last hour, I considered it being of interest to study the entire nocturnal activity and subsequent diurnal activity with 10 and 20 mg/kg b.w. as the effect may presumably remain during the active period. Here 10 mg/kg b.w. of reserpine showed 76 % of that of placebo in nocturnal activity and 100 % in diurnal activity. Between the 5th and 12th hour of nocturnal activity it was likewise 76 %. A reduction in nocturnal activity down to 49 % was reached by 20 mg/kg b.w., and in diurnal activity to 88 %. Between the 5th and 12th hour of nocturnal activity the value obtained was 31 %.

The results obtained corresponded with those of earlier investigations of reserpine, carried out by BROWN (24), LESSIN & PARKES (3), CAVIEZEL & BAILLOD (82) and others.

According to PLUMMER (88) the toxicity exceeds 1 000 mg/kg b.w. orally in rats.

I have also studied the lumi variant of reserpine (90), but it failed to give any response in doses up to 100 mg/kg b.w. orally. (The substance has been synthesized by Dr. Sixten Ljungberg, the State Pharmaceutical Laboratory and kindly been made available for the purpose of this investigation.)

Chlorpromazine chloride



LD₅₀: 485 (438—527) mg/kg b.w.

Doses: 2 mice per cage: 7.5 and 15 mg/kg b.w.

1 mouse per cage: 7.5 and 15 mg/kg b.w.

Potentiated narcosis 7.5 mg/kg b.w.: 118.4 ± 7.4 min.

Control: 47.2 ± 3.0 min.

df. 17 $P < 0.001$

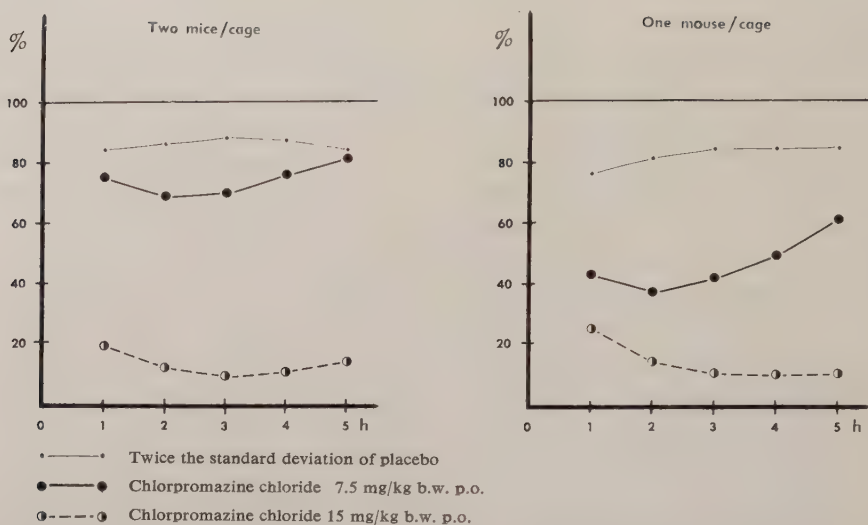


Fig. 21. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

A reduction exceeding twice the standard deviation was obtained in all the cases. The lower dose gave a greater effect with one animal per cage than with two, while the larger doses gave equivalent effects with one or two animals per cage. In contrast to reserpine the effect appeared already during the first hour to remain throughout the entire test period.

To investigate whether the effect would remain after the entire period of nocturnal activity, as with reserpine, the values of the high dose with one mouse per cage were compared with the placebo values during the last seven hours of the dark 12-hour period. The animals which had been given the drug showed a spontaneous activity amounting to 98 % of the placebo values. This probably means that no effect remained after 12 hours, in spite of the fact that the drug had produced a reduction of spontaneous activity of 10 % five hours after administration.

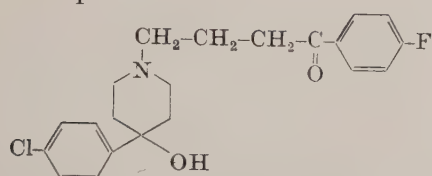
LESSIN & PARKES (3) and COOK ET AL. (13) have found a reduced spontaneous activity using the photo-electric method on mice, SHALLEK ET AL. (15) and SANDBERG (22) on white rats using jiggle cage, TRIPOD & GROSS (9) and JANSEN ET AL. (83) a reduced co-ordination with the fall-time method, and SANDBERG (22) with climbing test on mice. SHALLEK ET AL. (15) and SANDBERG (22) have in these cases given ED_{50} for chlorpromazine subcutaneously as 1.9 mg/kg b.w. and 4.2 mg/kg b.w., while JANSEN ET AL. (83) have given ED_{50} as 2.3 mg/kg b.w. subcutaneously.

CAVIEZEL & BAILLOD (82) have studied the effect of chlorpromazine

in doses between 0.5—64 mg/kg b.w. orally on spontaneous activity with mice using the earlier described combined apparatus (cf. p. 45) and obtained a dose-effect relation at which, as can be judged from their results, 16 mg/kg b.w. gave a reduction of spontaneous activity of about 50 %. LESSIN & PARKES (3) in an investigation of the ability of chlorpromazine to reduce the body temperature in mice give the maximum reduction as three hours after intraperitoneal administration.

COURVOISIER ET AL. (89) give LD_{50} as 75 mg/kg b.w. orally in mice, while SHALLEK ET AL. (15) give 530 mg/kg b.w., a value which corresponds better with that one found here.

Haloperidol



LD_{50} : 217 (175—270) mg/kg b.w.

Dose: 1 mouse per cage: 0.3 mg/kg b.w.

Potentiated narcosis 0.3 mg/kg b.w.: 41.9 ± 3.8 min.

Control: 42.8 ± 3.2 min.

df. 17 $P > 0.05$

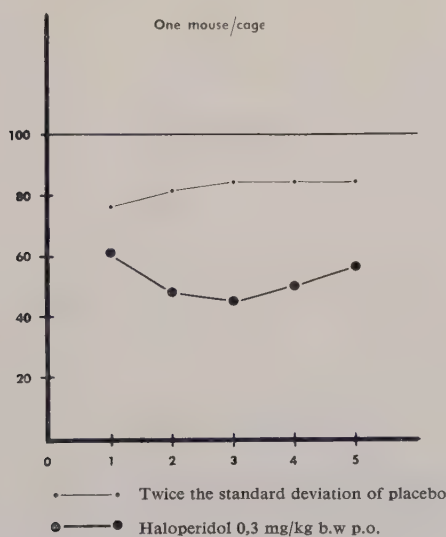
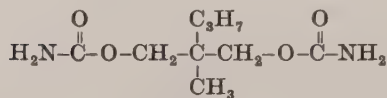


Fig. 22. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The substance which was synthesized and pharmacologically screened and compared with other substances of known sedative effect by JANSEN ET AL. (83) gave a reduction of spontaneous activity to 40 % of that of placebo in a dose of 0.3 mg/kg b.w. At the investigation carried out by JANSEN ET AL. (83) in which rotating rods were used, ED₅₀ was found to be 0.40 mg/kg b.w. Haloperidol had a quantitatively larger effect than chlorpromazine on spontaneous activity which also can be seen from Chapter V in which studies of the dose-effect on both preparations are indicated.

The dose administered gave no potentiated narcosis.

Meprobamate



LD₅₀ ~ 2 000 mg/kg

HD₅₀ = 445 (386 — 484) mg/kg b.w.

Doses: 2 mice per cage: 100, 200 and 400 mg/kg b.w.

1 mouse per cage: 100 and 200 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: 88.1 ± 6.3 min.

Control: 47.2 ± 3.0 min.

df. 18 *P* < 0.001

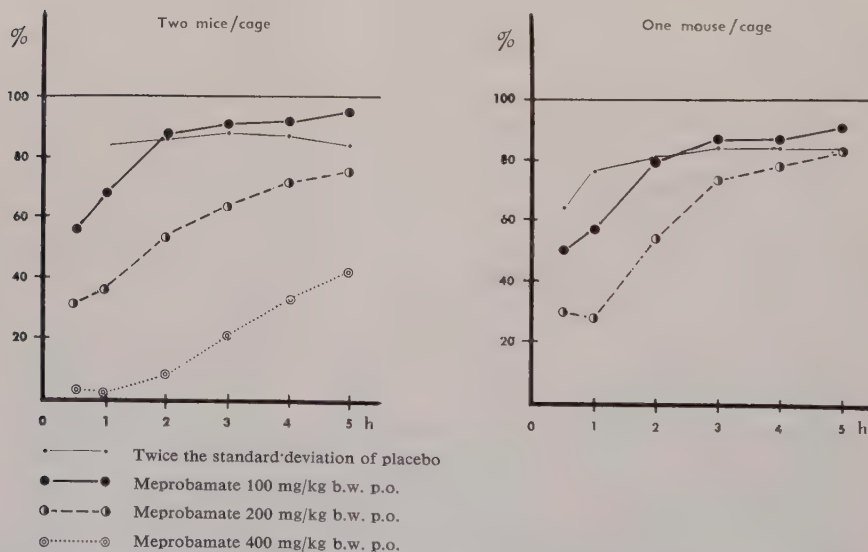


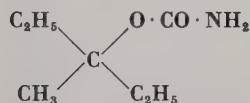
Fig. 23. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

To produce an effect, considerably greater doses were needed here than with the three preparations belonging to this group previously mentioned. The type of effect was also quite different. With the previous preparations, some time elapsed before the maximum effect was reached, but here the maximum effect could be observed even during the first half hour. After that the curve showed a marked positive inclination by contrast to the earlier ones, which means that the effect can be presumed to be of shorter duration. Study of the 60-minute periods shows in the case of the lower dose that the effect was present only during the first 60-minute periods. CAVIEZEL & BAILLOD (82) obtained increased spontaneous activity between 35 and 65 minutes after oral administration of a dose of 160 mg/kg b.w. and decreased in a dose of 200 mg/kg b.w.

SHALLEK (15) reports that in a jiggle cage study a subcutaneous dose of 240 mg/kg b.w. reduces the spontaneous activity of white rats to 50 %, while TRIPOD & GROSS (9), using the falltime method, obtained results showing reduced co-ordination with oral doses of 30—300 mg/kg b.w. JANSEN ET AL. (83) give ED_{50} as about 160 mg/kg b.w. subcutaneously with the same method. MELANDER (27) has studied spontaneous activity by the photo-electric method and compared the effect with that of emylcamate (see below) in oral doses of 50 mg/kg b.w. in mice and has noted reduced spontaneous activity.

Toxicity is given by BERGER (16) as 1 100 mg/kg b.w. in mice with oral administration, and by SHALLEK (15) as 2 000 mg/kg b.w. BERGER (16) states the dose producing paralysis in 50 % of the mice as 300 mg/kg b.w.; the HD_{50} obtained is thus higher than the paralytic dose found by BERGER (16).

Emylcamate



$LD_{50} > 1\,000$ mg/kg b.w.

Doses: 2 mice per cage 50 and 100 mg/kg b.w.

1 mouse per cage 100 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: 58.0 ± 7.0

Control: 42.8 ± 3.2

$df. 18$ $P > 0.05$

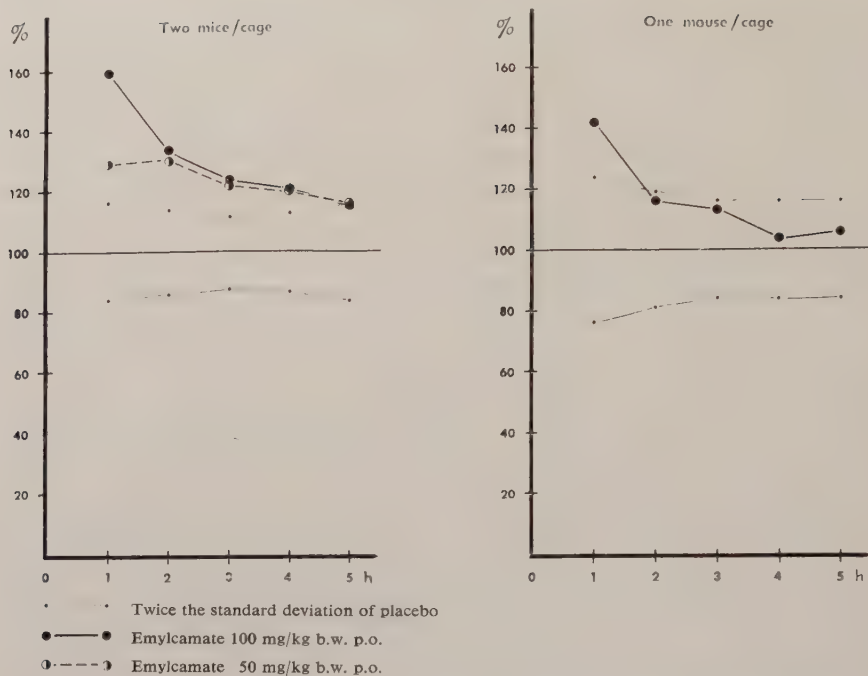


Fig. 24. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

Since this substance in earlier pharmacological studies (27) has shown properties which are similar in many respects to those of meprobamate, it would be interesting to examine its influence on spontaneous activity as determined by the method used here. In a study by MELANDER (27), this substance had produced a reduction in spontaneous activity which was greater than that produced by meprobamate; in this case the photo-electric method was used and equal doses of the two substances, orally administered, were compared.

The results obtained here, however, showed an entirely different trend. No reduction in spontaneous activity could be observed with either one or two animals per cage — on the contrary, there was stimulation. The results thus differed completely from those obtained from determination by the photo-electric method. MELANDER (27) gives LD_{50} as 550 mg/kg b.w. for i.p. administration to mice.

The dose of 100 mg/kg gave no potentiated narcosis (cf. meprobamate).

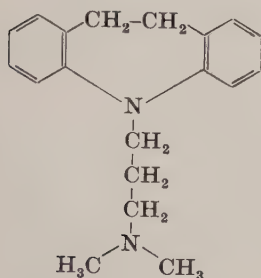
Anti-depressant drugs

The anti-depressant drugs tested were imipramine and iproniazid.

Imipramine was synthesized by SCHINDLER & HÄFLIGER (91) and shows some structural similarity to the phenothiazine derivatives. Pharmacological studies have been made by DOMENJOZ & THEOBALD (92), SIGG (93) and others.

Iproniazid was introduced as an anti-tubercular drug, but has subsequently been used for the treatment of depressive states. It has proved to be an active inhibitor of amino-oxidase and increases the excitatory effect of 5-hydroxy-tryptophane and 3,4-dihydroxy-phenylalanine but does not according to RANDALL & BAGDON (94) cause excitation in mice when administered alone in single non-lethal doses. PLETSCHER ET AL. (95) in a survey of monoamino-oxidase inhibitors state different effects on spontaneous activity.

Imipramine chloride



Imipramine

LD₅₀: 324 (291—389) mg/kg b.w.

Doses: 2 mice per cage: 15 and 50 mg/kg b.w.

1 mouse per cage: 15 mg/kg b.w.

Potentiated narcosis 50 mg/kg b.w.: 76.4 ± 7.8 min.

Control: 48.7 ± 2.8 min.

df. 18 $P < 0.01$

Since this substance shows a certain structural similarity to chlorpromazine and the following promethazine, doses of 15 mg/kg b.w. and 50 mg/kg b.w. were administered. As Figure 25 shows, the lower dose produced no reduction in spontaneous activity with either one or two animals per cage. The higher dose, however, did produce a reduction in spontaneous activity. The same result has also been obtained by SIGG (93), who reports that i.p. doses of 1—5 mg/kg b.w. to dogs, cats, rats and mice do not give any increase in the psychomotor test. However, 5—10 mg/kg b.w. did produce a sedative effect in cats and dogs, while higher doses were needed to produce sedation in rats and mice. DOMENJOZ & THEOBALD (92) have also obtained sedation in cats at these doses but were unable to detect any influence on spontaneous activity by the jiggle cage with 50 mg/kg b.w.

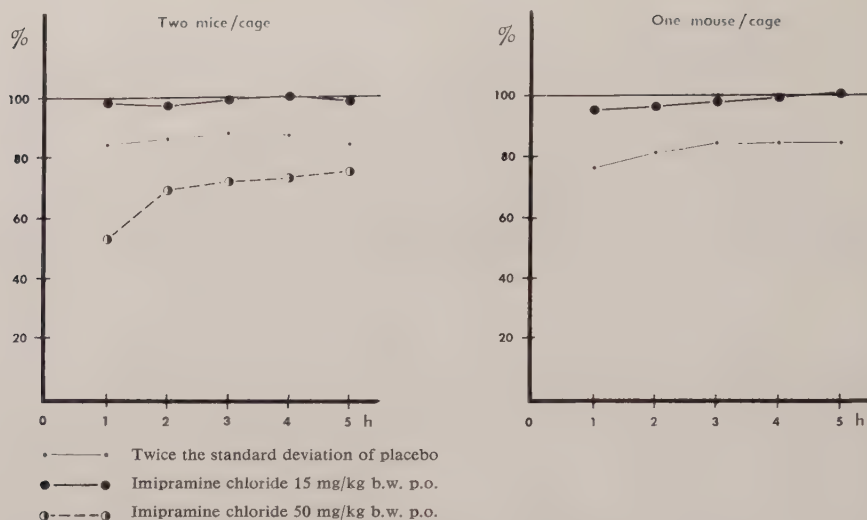
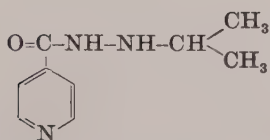


Fig. 25. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

subcutaneously to rats. ED_{50} was determined by the climbing test according to KNEIP (12) as 103 mg/kg b.w. for mice.

DOMENJOZ & THEOBALD (92) give the oral toxicity for mice as 400 mg/kg b.w. and the intravenous toxicity for the same animals as 35 mg/kg b.w.

Iproniazid



LD_{50} : 1 069 (909—1 165) mg/kg b.w.

Doses: 1 mouse per cage: 100 and 200 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: $274,5 \pm 27,0$ min.

Control: $68.5 \pm 4,1$ min.

df. 18 $P < 0.001$

The higher dose gave a reduction to about 60 % during the first half hour, while the other readings did not exceed twice the standard deviation. No direct stimulation of spontaneous activity was observable for aggregate values, but if the values for each period were studied the third and

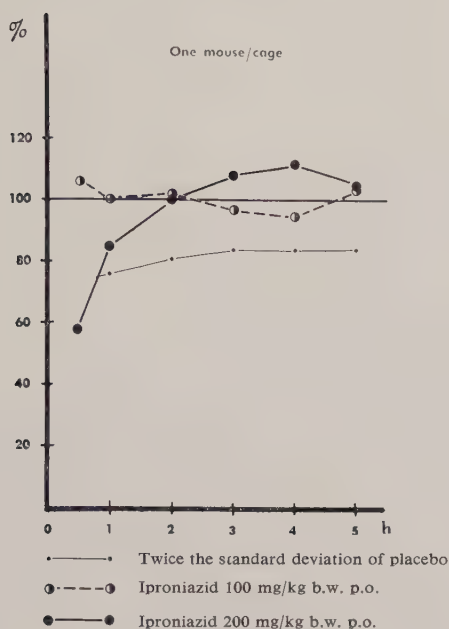


Fig. 26. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

forth hour gave a stimulation more than twice the standard deviation. BROWN (24) found a stimulation in lower doses and a reduction in spontaneous activity in higher doses by the photo-electric method. RANDALL & BAGDON (94) found no excitation when iproniazid was given alone in non-lethal doses. LD_{50} is given by RANDALL & BAGDON (94) as 959 mg/kg b.w. for oral doses to mice.

Strongly potentiated narcosis was obtained even with the lower dosage, although this dose did not produce any reduction in spontaneous activity.

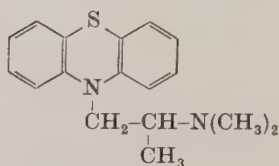
Antihistamines

This group often has a sedative effect in addition to the antihistamine effect and is therefore of interest in connection with the present systematic study. The similarity of structure between chlorpromazine and promethazine constituted a further reason why I have considered an investigation to be of interest.

Promethazine was first studied by HALPERN (96, 97, 98) who found antihistamine properties in this phenothiazine derivative.

Phenbenzamine like promethazine, has been studied pharmacologically by HALPERN (99) in a series of newly synthesized substances. It is generally described in pharmacological handbooks as having none or a mild sedative effect.

Promethazine chloride

*Promethazine*

LD₅₀: 315 (287—347) mg/kg b.w.

Doses: 2 mice per cage: 50 mg/kg b.w.

1 mouse per cage: 50 mg/kg b.w.

Potentiated narcosis 50 mg/kg b.w.: 119.3 ± 8.5 min.

Control: 62.2 ± 5.8 min.

df. 18 $P < 0.001$

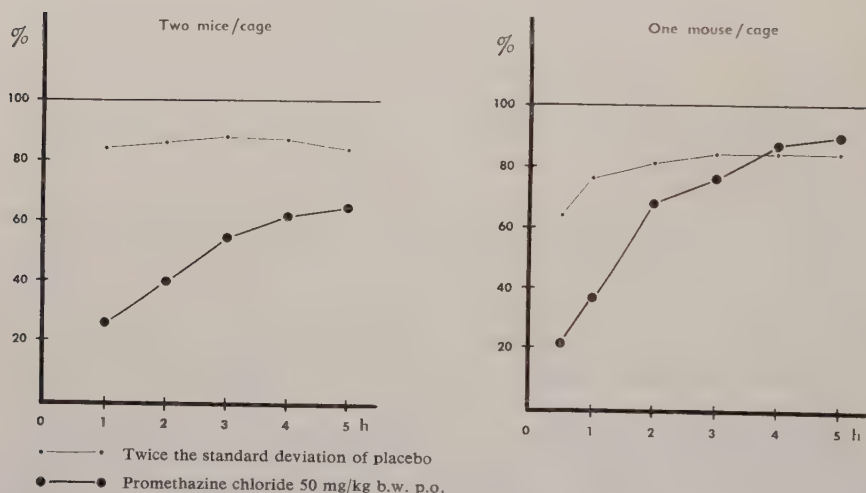


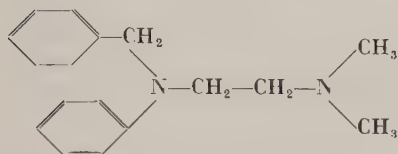
Fig. 27. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The dose administered was more than three times greater than the maximum administered dose of the structurally similar chlorpromazine. Even so, the effect on spontaneous activity was considerably less than with the latter. A marked effect was obtained particularly during the first hour, but this gradually decreased. This also represents a departure from the effect of the chemically related chlorpromazine. SCHMITERLÖW (100) using the autoradiographical method, found that S³⁵-labelled promethazine

was recognized in the mouse brain in a high concentration one hour after administration but reduced after a five hour period.

SANDBERG (22) in his investigations of phenothiazine derivatives did not obtain any effect on spontaneous activity in white rats with subcutaneous doses of promethazine of up to 300 mg/kg b.w. when using the jiggle cage, but on the other hand the climbing test according to KNEIP (12), when used in the same investigation, showed an effect which was greatest during the first hour.

Phenbenzamine chloride



Phenbenzamine

LD₅₀: 269 (249—302) mg/kg b.w.

Doses: 2 mice per cage: 50 mg/kg b.w.

1 mouse per cage: 50 mg/kg b.w.

Potentiated narcosis 50 mg/kg b.w.: 40.1 ± 3.7 min.

Control: 32.5 ± 3.2 min.

df. 18 $P > 0.05$

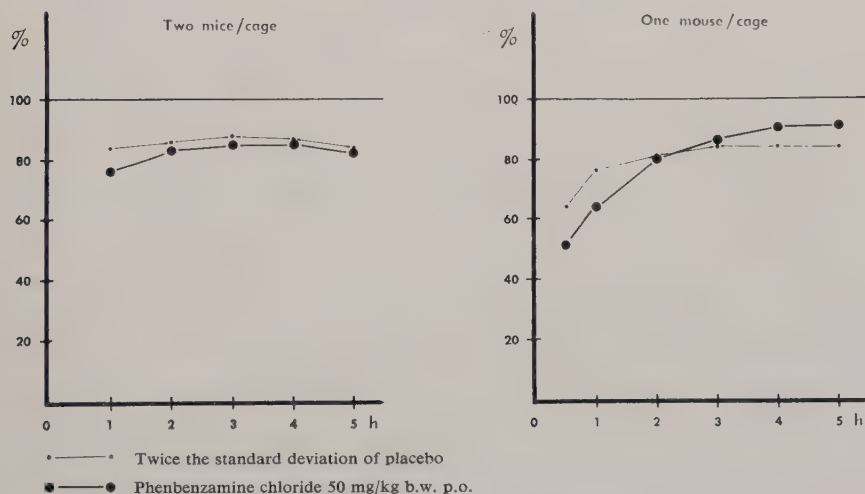


Fig. 28. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

A reduction in spontaneous activity more than twice the standard deviation was produced during the first hour. The effect was however of a different order of magnitude than with promethazine, and this was also true of potentiated narcosis, which was not determinably prolonged. HALPERN (99) gives the orally LD_{50} for rats as 300 mg/kg b.w.

In a serie of antihistamines BJERVER & GOLDBERG (101) found central depressive effects of both phenbenzamine and promethazine with flickery fusion and blink reflex test.

Analgesic and antipyretic drugs

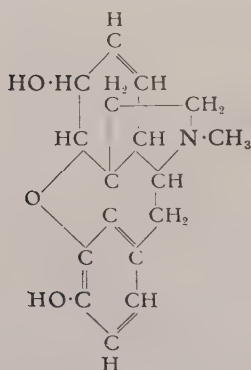
Analgesics also belong to the group of central depressants, and I therefore found it to be of interest to include this group too. I have studied one representative of the strong analgesics, namely morphine, as well as acetylsalicylic acid and phenazone among non-narcotic analgesics.

Morphine has previously been studied in the course of several investigations of spontaneous activity (8, 26, 42, 82, 102), but neither acetylsalicylic acid nor phenazone (40) have received much attention in this connection.

These investigations are made with only one animal per cage.

Strong analgesic:

Morphine sulphate



Morphine

$LD_{50} \sim 1\,500$ mg/kg b.w.

Doses: 1 mouse per cage 10 and 50 mg/kg b.w.

Potentiated narcosis 10 mg/kg b.w.: 31.9 ± 2.6 min.

Control: 26.1 ± 3.3 min.

df. 18 $P > 0.05$

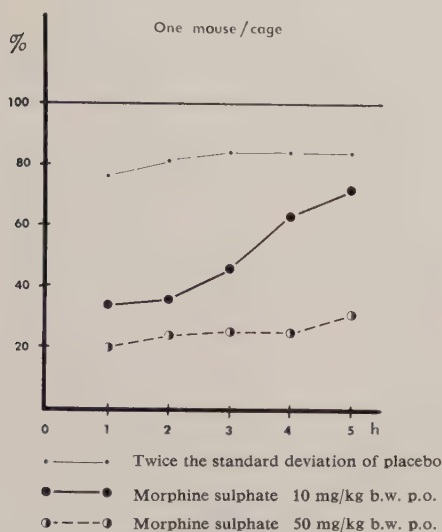
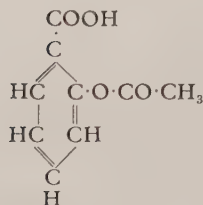


Fig. 29. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

Both doses gave a reduction in spontaneous activity which persisted throughout the test period. CAVIEZEL & BAILLOD (82), using the combined apparatus described previously (p. 45), obtained an increased activity in mice with 18 mg/kg b.w. administered subcutaneously. FORST (42) reports a reduction in the spontaneous activity of mice on a tipping plate with doses of 0.1–10 mg/kg b.w., while s.c. doses of 50 and 100 mg/kg b.w. produce stimulation. FÜHRER & FELDHOFFEN (102) using similar apparatus, obtained a reduction in spontaneous activity with doses of 0.1 and 1 mg/kg b.w., but an increase with 10 mg/kg b.w. administered s.c. Increased spontaneous activity in mice measured by the photo-electric method has been obtained by WINTER & FLATAKER (26) with 25 mg/kg b.w. administered subcutaneously and by NIESCHULTZ ET AL. (8) with 10 mg/kg b.w. given intraperitoneally.

Non-narcotic analgesics:

Acetylsalicylic acid



$\text{LD}_{50} \sim 1\,700 \text{ mg/kg b.w.}$

Dose: 1 mouse per cage: 300 and 500 mg/kg b.w.

Potentiated narcosis 500 mg/kg b.w.: $39.1 \pm 2.9 \text{ min.}$

Control: $32.5 \pm 3.2 \text{ min.}$

$df. 18 \text{ } P > 0.05$

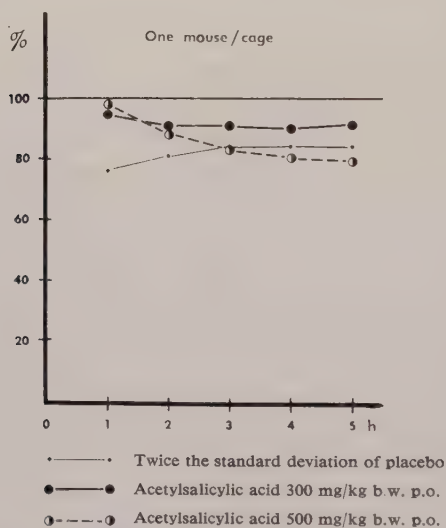


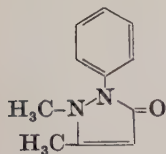
Fig. 30. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

Both dosages produced a slight reduction in spontaneous activity, but with the lower dose this did not exceed twice the standard deviation. The higher dose, however, gave an effect which barely exceeded this limit during the last two hours.

To determine the influence of the higher dose on normal body temperature, this was measured in 10 mice over a two-hour period, readings being taken every 30 minute. Comparison with a control group of 10 mice did not show any significant difference ($0^{\circ}.35 \pm 0^{\circ}.25$; $t = 1.41$ *df.* 18 $P > 0.05$). The temperature drop with acetylsalicylic acid was greatest after 90 minutes, i.e. $0^{\circ}.90 \pm 0^{\circ}.17$ C, while the control group at the same time showed a temperature drop of $0^{\circ}.55 \pm 0^{\circ}.18$ C.

As can be seen above, no potentiated narcosis was obtained.

Phenazone



$LD_{50} > 1\ 000$ mg/kg b.w.

Doses: 1 mouse per cage: 100 and 300 mg/kg b.w.

Potentiated narcosis 300 mg/kg b.w.: 123.0 ± 8.5 min.

Control: 48.7 ± 2.8 min.

df. 18 $P < 0.001$

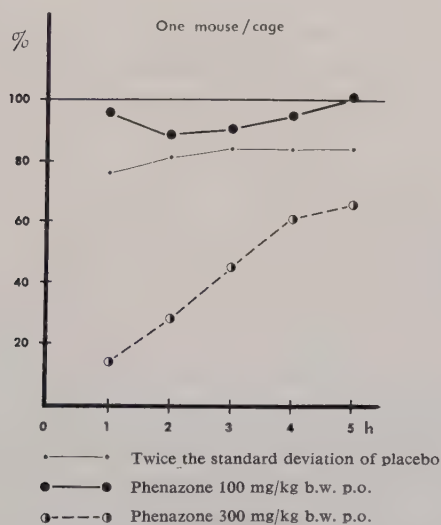


Fig. 31. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The lower dose produced no effect, while the higher one gave a sharp drop in spontaneous activity, which was most marked during the first hour. This dose also gave a marked decrease in body temperature measured in the same way as for acetylsalicylic acid. Thus at 30 minutes, when the temperature drop was greatest, body temperature was $4.7 \pm 0.23^\circ\text{C}$ below that of the control group ($t = 20.5$ *df.* 18 $P < 0.001$). FORST (40), using smoked paper, obtained a reduction in spontaneous activity in mice with s.c. doses of 10, 50 and 100 mg/kg b.w.

In contrast to the previous drugs, a sharp increase in hexobarbital narcosis was also obtained.

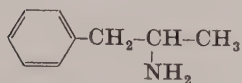
Central Stimulants

As has already been pointed out, the study of central stimulant drugs should take place during the mice's less active period, i.e. diurnal activity; this was the case both in GARBERG and SANDBERG's jiggle-cage study of rats (103) and DEWS' study of mice by the photo-electric method (51).

The nocturnal activity should be close to optimum, since the mice derive pleasure from this apparatus, and therefore it may be difficult to stimulate them further. The primary object of the present investigation, has been to study drugs which are regarded as having a sedative effect in therapy. I found however that it would be interesting to study the effects of amphetamine and caffeine under the same conditions as for central depressant drugs i.e. during nocturnal activity.

In a preliminary investigation employing the cross-over method described in Chapter I, the influence of amphetamine, diethylpropione and caffeine on diurnal spontaneous activity was also studied.

Amphetamine sulphate



Amphetamine

LD₅₀: 41 (25—67) mg/kg b.w.

Doses: 2 mice per cage: 10 mg/kg b.w.

1 mouse per cage: 10 mg/kg b.w.

Potentiated narcosis 10 mg kg/b.w.: 30.0 ± 0.5 min.

Control: 32.5 ± 3.2 min.

df. 18 $P > 0.5$

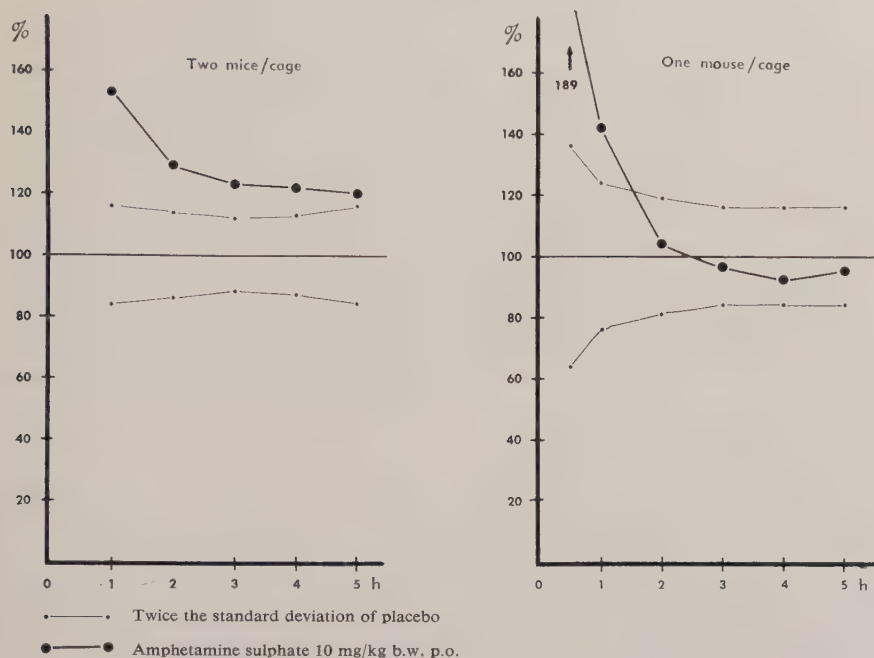


Fig. 32. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

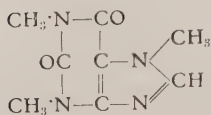
The preparation was administered in fairly large dose to see whether any toxic influence resulting in reduced spontaneous activity could be observed. The results showed an increase in spontaneous activity with both one and two animals per cage. This increase was greatest during the first hour.

MELANDER and GLINIECKE (104), in their studies employing the same method, obtained similar results in a comparative investigation of tranylpromine and diethylpropione.

As has been demonstrated by GUNN and GURD (105), amphetamine gave greater excitement and CHANCE (106) found a higher toxicity if amphetamine was administered to groups of animals than if only one animal occupied each cage. It would be interesting to examine the effect if several animals were kept in the same cage during the experiment. The same dose, 10 mg/kg b.w. was therefore administered to four mice per cage and the values found showed a reduction instead of an increase of the estimated activity.

It has to be pointed out that in the toxicity test I have, as for every other substance, used five animals per cage.

Caffein sodium salicylate

*Caffein*

LD₅₀: 522 (479—589) mg/kg b.w.

Doses: 1 mouse per cage 50 and 100 mg/kg b.w.

Potentiated narcosis 100 mg/kg b.w.: 43.4 ± 3.6 min.

Control: 50.3 ± 2.7 min.

df. 17 $P > 0.05$

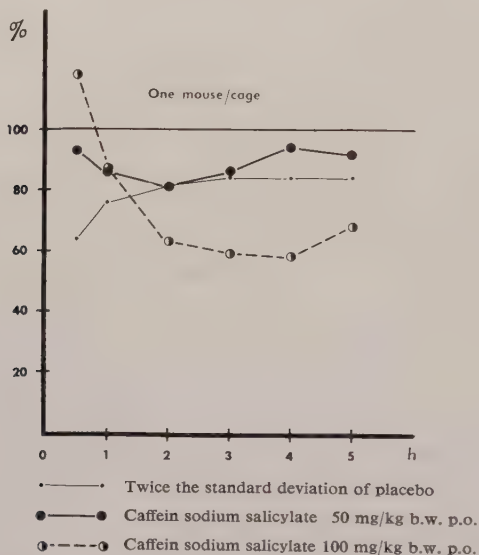


Fig. 33. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

The results obtained were quite unexpected. At the times of taking readings normally adhered to in the course of the investigation, i.e. from 60 minutes onwards, the values for spontaneous activity were in fact lower than those with placebo. Similar results were obtained with corresponding doses of caffeine itself, suspended in 0.1 % modocoll® solution.

Studies of diurnal activity

Amphetamine 10 mg/kg b.w. produced an increase in spontaneous activity which as greatest exceeded three times the placebo. Diethylpropione under the same conditions and in the same dosage produced an increase which was somewhat less, but still more than twice as great as the spontaneous activity obtained on administration of placebo. After computation by *t*-analysis, both amphetamine (10 mg/kg b.w.) and diethylpropione (10 mg/kg b.w.) showed a significant difference from placebo at $P = 0.05$. On the other hand, neither amphetamine in a dose of 5 mg/kg b.w. nor caffeine gave effects which could be definitely shown to differ from the placebo values.

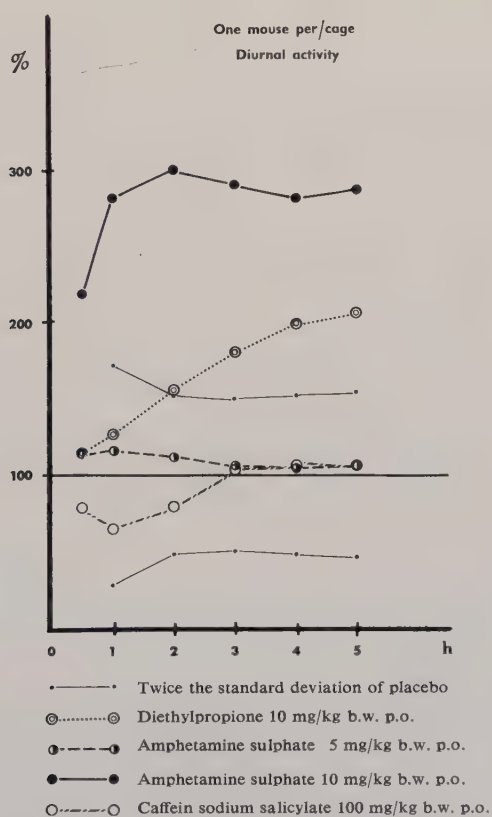


Fig. 34. Spontaneous activity in % of placebo for aggregate values during a five hour test period.

Long-term test

To study whether the administration of drugs over a long period affected the spontaneous activity of mice, the placebo values were recorded throughout the 12-hour period of darkness with two mice per cage for about one month. During this period the same animals were given a drug every second day, in all 11 different drugs, administered at random. The regression was rectilinear but the slope was not significant ($b = -14.3 \pm \pm 32.7$; $t_b = 0.44$ *df.* 260 $P > 0.6$). Each point represents the mean of about 25 placebo values.

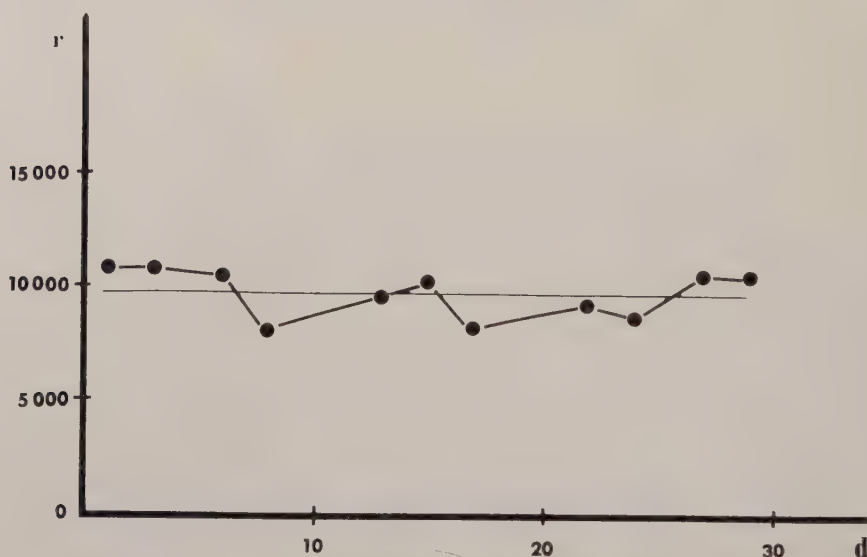


Fig. 35. Placebo values recorded throughout the 12-hour period of darkness with two mice per cage for about one month. The animals were given a drug every second day, in all 11 different drugs. Each point represents the mean of about 25 placebo values.

As Table 11 shows, the relative standard deviation for the placebo values used for direct comparison with active drugs were somewhat higher than the values where no drugs were given (cf. Table 7), but no significant difference could be established.

Only in exceptional cases more than five drugs were tested on the same mice. Where this was done, it was with drugs having a mild effect on spontaneous activity.

Reference may also be made to the results mentioned earlier (cf. p. 54) where administration of 15 mg chlorpromazine per kg b.w. gave a reduction

Table 11. Comparison between placebo values when no drug (I) or drugs were given every second day (II).

(Placebo values in first row are taken from Table 7)

	60 min.	120 min.	180 min.	240 min.	300 min.
Placebo I	1 143 $\pm$ 23	2 565 $\pm$ 47	3 855 $\pm$ 63	4 998 $\pm$ 87	6 114 $\pm$ 126
S%	7.9 $\pm$ 1.4	7.1 $\pm$ 1.3	6.3 $\pm$ 1.2	6.7 $\pm$ 1.2	8.0 $\pm$ 1.5
Placebo II . . .	1 195 $\pm$ 41	2 529 $\pm$ 82	3 793 $\pm$ 124	5 014 $\pm$ 164	6 064 $\pm$ 201
S%	11.5 $\pm$ 2.5	10.7 $\pm$ 2.3	10.8 $\pm$ 2.3	10.8 $\pm$ 2.3	11.0 $\pm$ 2.4
Diff. S%	3.6 $\pm$ 2.9 <i>df</i> 24 <i>P</i> > 0.2	3.6 $\pm$ 2.6 <i>df</i> 24 <i>P</i> > 0.1	4.5 $\pm$ 2.6 <i>df</i> 24 <i>P</i> > 0.05	4.1 $\pm$ 2.6 <i>df</i> 24 <i>P</i> > 0.1	3.0 $\pm$ 2.8 <i>df</i> 24 <i>P</i> > 0.2

in spontaneous activity to 10 % of placebo during the fifth hour. During the following seven hours of the dark period the animals showed a spontaneous activity amounting to 98 % of placebo. Reserpine, on the other hand, gave effects which persisted throughout the dark period (cf. p. 53).

DISCUSSION

OUR knowledge of the relation between chemical structure and biological effect is as yet in part very limited. In the search for new drugs one must therefore continue to apply pharmacological screening in order to test the innumerable substances which are continuously synthesized by the chemists.

The screening test only has to provide answer to the question if a substance is of interest or not. In order to determine the degree of interest, follow-up studies concerning dose-effect relationships and quantitative assays are necessary.

Naturally it is desirable to get as much information about a substance as possible in the screening test. However, the problem involved in developing a new drug is extremely complex because the effects and side-effects only are discovered when the compound is studied in detail.

In order to determine whether a substance is of interest or not it is necessary to be able to measure an effect that is better or equal to that produced by the control substance. This can be inactive (placebo) or can have a certain known effect.

In studies of spontaneous activity the normal activity has also been used as a comparison, but without any inactive substance being given to the test animals. However, saline or the vehicle in which the substance has been dissolved or suspended is widely used as a control substance in order to minimize the sources of error.

It is not enough when the effect just exceeds that obtained with saline, as it is known that a biological variation always exists. The greater this variation, the more difficult it is to decide whether there is an effect or not with the substance tested.

The method described by LJUNGBERG (37) is too time-consuming for the screening of drugs having an assumed sedative effect as it required at least 15 days. Primarily it is intended for further studies of substances. I have therefore chosen to examine whether this apparatus can be used for screening with the method described in Chapter I. As can be seen from the previous chapter, the normal variation of saline orally administered was satisfactory both with one and two animals per cage. Consequently, the testing of known drugs should be decisive for the suitability of the method, also whether one or two animals per cage should be used in the experiment. These tests should also give the limits which should be exceeded to establish whether the substance would be of interest or not. Preference should be given to the method which gives the greatest reduction in spontaneous activity. It is possible that various types of drugs give different responses according to the number of animals used, especially as with the photoelectric method BROWN (107) considers that sedatives and hypnotics could be distinguished from tranquilizers by their different effects on spontaneous activity if one or a group of animals is used.

Where twice the standard deviation has been set as a limit, all the tested substances at the highest dosage levels, with the exception of central stimulants, other than emylcamate caused a reduction in spontaneous activity and would thus be of interest for further investigation. Also of interest is emylcamate, as this drug produced during the first hour a stimulating effect exceeding the standard deviation three times both for one and two animals.

Of the doses administered to two animals per cage this limit would mean that further tests with methylpentynol 200 mg/kg b.w., amobarbital 50 mg/kg b.w. and imipramine 15 mg/kg b.w. would not be of interest.

If the limit is increased to three times the standard deviation then this limit would not be exceeded by giving 200 mg/kg b.w. of carbromal, 100 mg/kg b.w. amobarbital, and 200 mg/kg b.w. thalidomide and 50 mg/kg b.w. phenbenzamine lay just at the limit.

Where there is one mouse per cage and twice the standard deviation is set as the limit, the administration of 1.5 mg/kg b.w. of ethylalcohol, 50 mg/kg b.w. amobarbital, 15 mg/kg b.w. imipramine, 100 mg/kg b.w. iproniazid, 100 mg/kg b.w. phenazone and 300 mg/kg b.w. acetylsalicylic acid would not fulfil the demand for further investigation. However, in higher dosages these substances brought about a reduction which was more than twice the standard deviation. Imipramine was only given in the small dose, but with two mice per cage 50 mg/kg b.w. produced a reduction which was more than twice the standard deviation; judging from the experience of other experiments the same results would probably have been obtained in the case of one animal per cage.

If three times the standard deviation is used as the limit, then the reduction in spontaneous activity brought about by 200 mg/kg b.w. of methylpentynol, 100 mg/kg b.w. thalidomide, 200 mg/kg b.w. iproniazid and 500 mg/kg b.w. acetylsalicylic acid would not exceed this limit.

With one mouse per cage a greater reduction of spontaneous activity at the same dose level was obtained almost throughout. Thus one mouse per cage is to be preferred for a screening test using the method described in Chapter I, particularly as this involves both less labour and lower costs for animals. Furthermore, the time factor in administration will play a minor role for the early readings. Here the time difference between the administration of the substance to the first and the last animals amounts from five to ten minutes. Naturally with two animals per cage the time difference is twice as great and thus of major importance with a reading taken as early as 30 minutes afterwards.

By noting the readings in each individual case in the experiment one can also calculate whether there is a definite difference between placebo and a substance. However, this is perhaps of even greater value if it is desired to compare two substances and by doing so to examine whether there is a difference in effect at a certain dose level. An example of this is the effect of the ectylurea compared with two of the ureids which have been clinically used for a long time, namely bromural and carbromal. In both the cases ectylurea showed a significantly larger reduction of spontaneous activity. Using the photo-electric method, a good effect has been obtained with ectylurea by KINNARD & CARR (11) and WEAVER & MIYA (74). On the other hand, they found little or no effect on co-ordination with corresponding doses when determined by the rotating rod method.

When studying the results of the different groups it can be seen that of the large group *hypnotics and sedatives*, only chloral hydrate of the aldehyds has been tested and this has given the most marked effect during the first

hour. The two alcohols — ethylalcohol and methylpentynol — also showed maximum effect during the first hour.

A marked maximum effect during the first hour has also been obtained with hexobarbital, amobarbital and glutethimide.

A reading during the first two hours after administration should be sufficient for all the substances belonging to the group hypnotics and sedatives in order to decide whether the substance is of interest or not. From the results obtained it appears that the doses administered must be between 10 % and 25 % of LD_{50} if the reduction is to be more than twice the standard deviation, except for ectylurea and thalidomide, both of which have low, acute toxicity and a hypnotic level which is close to the median lethal dose (LD_{50}). FERGUSON & LINN (71) have postulated that ectylurea clinically had a wide range between sedation and sub-hypnosis, while PINDELL ET AL. (69), FANCHER & LIM (71) and LJUNGBERG & STRÖM (70) have all demonstrated the large »sedative» range of the substance used in experiments on animals.

As to the other substances belonging to this group it was most noticeable that to obtain a reduction of spontaneous activity more than twice the standard deviation required doses close to the hypnotic dose (HD_{50}). This was particularly the case with methylpentynol, hexobarbital, amobarbital and glutethimide. These too showed a maximum effect during the first 30 minutes, and are mainly used in therapy as hypnotics.

The high-melting of phenylcrotonylurea, which had not previously been studied pharmacologically, differed from the ethyl compound as it had its greatest effect during the first hour. Here it is of interest that the low-melting geometric isomer did not produce any reduction, in fact it stimulated spontaneous activity during the first 60-minute period.

Tranquilizers are often classified as major or minor tranquilizers, e.g. reserpine and chlorpromazine belong to the former sub-division while meprobamate belongs to the latter. Haloperidol may well be considered a major tranquilizer and emylcamate as a minor tranquilizer, but more information and study are perhaps necessary for the further classification of these drugs.

Reserpine did not have its maximum effect until after five hours and thus showed the importance of having sufficient time to make readings in experiments of this type. Figure 20 shows the importance of having the opportunity to use the values obtained from each period of time. The long onset also coincides with those of earlier experiments with reserpine (3, 4, 88). As the toxic dose exceeded 1 000 mg/kg b.w., the dose which produced a reduction of spontaneous activity more than twice the

standard deviation was far from LD_{50} . In the present experiments reserpine did not produce any potentiated narcosis with doses of 10 mg/kg b.w. despite the fact that hexobarbital was not administered until 120 minutes after the reserpine. This shows the importance of using several methods for the determination of a sedative effect. This question will be discussed more in Chapter VI.

Chlorpromazine also gave a reduction of spontaneous activity which was more than twice the standard deviation with doses far below LD_{50} . This was also noted with haloperidol which has the same qualitative but greater quantitative effects than chlorpromazine according to JANSEN ET AL. (83). Also the reduction of spontaneous activity with chlorpromazine and haloperidol showed a certain similarity in induction time and duration.

Meprobamate had quite a different effect on spontaneous activity than the above-mentioned three drugs and was more like the preparations examined in the group sedatives and hypnotics, such as methylpentynol, glutethimide and amobarbital, all of which produced a maximum effect during the first hour and which rapidly disappeared. Some authors (108, 109) are more inclined to place meprobamate in this group than to consider it a tranquilizer.

Emylcamate produced an increase in spontaneous activity instead of a reduction, and the results thus differ from those obtained with the photo-electric method by MELANDER (27). With as high single dose as 1 200 mg/kg b.w. there was no decline in performance in the psycho-technical experiments carried out by JONSSON & ANDERSÉN (110). Consequently these tests on man correspond fairly well with the results obtained here.

Imipramine belongs, according to BENSON & SCHIELE (111) to the central nervous system suppressants and is considered to have sedative properties similar to those of the phenothiazines. At the dose level where chlorpromazine brought about a reduction of spontaneous activity down to 10 % of that of placebo no effect was obtained with imipramine, while when the dose was increased to 50 mg/kg b.w. the reduction was more than twice the standard deviation as was the case with promethazine. DOMENJOZ & THEOBALD (92) by applying the climbing test, which requires a certain initiative of the animals have also obtained a reduced activity.

Ipreniazid has not given any reproducible effect with the low dose, whereas this was brought about with the higher dose. The reducing effect reached its maximum during the first half hour and was followed by a stimulation during the third and fourth hour. If this stimulation was real or a compensatory increase after the initial reduction is difficult to say. Varying results have been reported from the experiments on animals, causing stimulation,

reduction or having no effect at all on spontaneous activity. The reason for these varying results has not yet been made clear, reports PLETSCHER ET AL. (95).

Of the antihistamine preparations both promethazine and phenbenzamine produced effects which were more than twice the standard deviation and being greatest in the first hour. However, on the same dose level promethazine produced a greater reduction than phenbenzamine. As is evident from the experiments of SANDBERG (22), no effect has been obtained with promethazine in the jiggle cage method, though the climbing test has had effect.

Within the analgetic group, morphine in doses of 10 and 50 mg/kg b.w. caused reductions which were more than twice the standard deviation. The results showing increased spontaneous activity with high doses in several experiments on animals (8, 26, 42, 82, 102) were thus not obtained from the doses and with the apparatus used here. Either the doses were not sufficiently high or otherwise the ability was lost for a regular pattern of movements which is required for this apparatus.

Phenazone 300 mg/kg b.w. and acetylsalicylic acid 500 mg/kg b.w. gave both a reduction which was more than twice the standard deviation, while the lower doses 100 and 300 mg/kg b.w. did not produce an effect which made them of interest for further testing of the sedative effect. The interesting fact was that 300 mg/kg b.w. of phenazone gave a marked reduction and with the same dosage a significant potentiated hexobarbital narcosis, while acetylsalicylic acid barely exceeded twice the standard deviation and with the same dose did not produce any potentiation of the hexobarbital narcosis. In the same way phenazone caused a definite drop in temperature, which was not produced with acetylsalicylic acid. LESSIN & PARKES (3) have shown with chlorpromazine and reserpine that there is a relationship between the drop in temperature and the reduction of spontaneous activity. This is the same with phenazone in these experiments.

The effects of two stimulants on spontaneous activity during nocturnal activity has been investigated, in spite of that it is less suitable to study these preparations during the active period of the animals, as the activity of the animals during this period probably closely corresponds to the maximum.

As with this type of apparatus it can be expected that a toxic dose will produce a reduction in spontaneous activity, a rather high dose of amphetamine was administered. However, both with one and two animals per cage this dose gave an increase of spontaneous activity, which reached its maximum during the first 30-minute period.

Caffein was administered in the form of its sodium salicylate and gave

unexpected values. The first 30-minute period somewhat exceeded the placebo values, but during the remaining test period there was a *reduction* in spontaneous activity.

Increases in spontaneous activity were obtained with the turntable (82), the photo-electric method (51), jiggle cage (20), the sand method (41) and by a photographic method used by SCHAUMANN & STOEPEL (112). With all these methods the activity of the mice moving about in the cage was registered. With the apparatus, used by me, a certain desire and initiative is needed for the animals to jump into the wheel and run in it. It would be of interest to use a combination of the photo-electric method and this type of squirrel wheel in order to get an idea of the behaviour pattern of the animals, an arrangement which could be easily done by mounting photo-electric cells and red lamps near the bottom of the cage. Red lamps should be used as red light cannot be perceived by mice; this is important in studying the nocturnal activity in mice.

In the previously described screening-test method all the substances with known sedative effects have produced a reduction of spontaneous activity of more than twice the standard deviation when the substance was administered in doses of up to 20 % of LD_{50} .

Naturally other substances may also produce a reduction of spontaneous activity. Here can be cited drugs which have a peripheral muscle-relaxing effect and other causing co-ordination disturbances as well as preparations which in one way or the other cause discomfort to the mice. As has been emphasized before, in a screening test it is more important not to lose any substance which might be of interest than to include a substance which further tests would show did not have the presumed effect. If the substance is administered in a dose which is at a safe distance from LD_{50} and produces a reduction in spontaneous activity which is more than twice the standard deviation, the substance is probable to have a pharmacodynamic effect of some kind.

From the above it can be realized that the choice of dose is of great importance. For the screening of stimulating substances 10 and 50 % of LD_{50} are recommended by DEWS (51). However, in sedative effect tests preparations which do not have any effect in doses of about 20 % of LD_{50} using this method are probably not of interest in this respect. Almost all pharmacologically active substances in doses as high as 50 % of LD_{50} would mainly with this method bring about a reduction of spontaneous activity.

However, in a screening test the problems of choice of dose may not be so great because, as a rule, an approximate LD_{50} has been determined

beforehand. A usual method when one wishes to investigate a series of substances is then to determine a suitable relationship value. The results obtained here show that it is possible to arrive at quite different relationship values with different groups of drugs having a sedative effect. The classical hypnotics and sedatives require larger doses than major tranquilizers.

As to the amount of the doses given, of theoretical interest is the comparison of the doses on man made by LJUNGBERG (113). Using the Gaussian formula he found a good relation between the doses in mice, used by me, and the average clinical doses on human beings. To arrive at suitable relationship values, when going over from experiments on animals to clinical tests, is obviously of both practical and theoretical importance.

A reading during the first two hours has usually been sufficient for the reduction to be at least twice the standard deviation. However, to prolong the reading time during a five hour period and taking readings every half hour is probably worthwhile as this may give a certain idea of the onset and duration of the substance. Moreover, preparations which have a late effect are not lost with this method. In order to be able to study these effects, one should utilize spontaneous activity during each period also for preparations which have a late effect, as these may otherwise be lost as the aggregate value gives spontaneous activity only from the beginning of the test.

Figure 20 which concerns reserpine, shows the importance of using a longer test period. Even for preparations with a transient effect it may be valuable to avoid too short reading times or that readings are taken only on certain occasions. The importance of this can be seen from the experiments with methylpentynol, amobarbital, glutethimide and meprobramate. Obviously no important conclusions about the effect of the preparations can be made, but these data can naturally be of value for the continuous studies of dose-relationship and comparative quantitative determinations (cf. Chapter IV and V).

LJUNGBERG (37) in his experiments used two animals per cage. However, my experiments showed that with this method it is probably advantageous to use only one animal per cage, as in almost all such cases a greater effect was obtained with the same dose level as when two animals per cage were used.

For practical reasons the limit for deciding whether further experimentation would be of interest or not is based on the previously determined standard deviation for placebo over a 15-day period. As the placebo values used for comparison refer to placebo values obtained when drugs and placebo have been administered alternatively, this standard deviation may

be greater; likewise the spontaneous activity of the mice may be affected during such a period. These experiments showed that this was not the case, despite the fact that the assessment was made during the period when the largest amount of drugs was administered. Neither could there be noted any significant difference in the standard deviations of the placebo values and those previously determined, nor any definite decrease in spontaneous activity of the animals, despite the fact that on each occasion an active drug was administered. Thus it is very unlikely that any of the preparations previously administered would have produced an effect that would influence the next substance as the metabolism of mice is rapid and they were not given the preparation more often than every second day. If a preparation causes a marked reduction or a long-lasting effect on spontaneous activity, the animals should obviously be replaced by others. In these experiments the same mice have been given, as a rule, five preparations at the most.

The method used was labour-saving as it took less than 15 minutes a day to administer the drugs and thus this work did not encroach on other tasks. Photographs automatically taken of the readings also saved labour. If a tripple cross-over test with placebo and two doses of the substance were preferred, altogether 45 minutes were required for administration. The fact that the mice had a relatively constant spontaneous activity for a period of at least five hours made it possible to extend the test period over five hours. This was an advantage with substances having a late effect and also it gave an idea of the onset time and the duration of the substance.

The preliminary experiments with stimulating drugs during diurnal activity gave expected results as far as amphetamine and diethylpropione were concerned, while caffeine caused a reduction. However, the reduction was not of such magnitude to equal twice the standard deviation.

As the excitation and toxicity of amphetamine has proved to be considerably greater when used on groups of animals than on one animal (105, 106), I considered it to be of interest to make an estimation of spontaneous activity with four mice per cage as well. However, no increase in spontaneous activity was obtained here; instead there was a reduction. Also here, as with caffeine, it would be of interest to carry out comparative experiments with a combination apparatus, which I have earlier suggested, using photo-electric cells and squirrel wheel in the same cage.

Further studies of stimulating drugs with this apparatus would be of interest, as it would probably be possible to distinguish which substances produce excitation with disturbances in co-ordination and behaviour from those substances producing stimulation without upsetting the pattern of movement and initiative of the animals.

CHAPTER IV

ESTIMATION OF THE LOG DOSE RESPONSE LINE

THE existence of a linear dose-response relationship is generally a prerequisite for quantitative determinations. If linearity exists in a given range, it should be possible to study substances presumed to have the same mode of action quantitatively by a four-point assay in this range of response.

LJUNGBERG (37) has shown that the experimental apparatus gives linearity for phenobarbital sodium in the dose-range 9.5—80 mg/kg b.w. with subcutaneous administration, provided that a log-log scale is used. As has already been mentioned, this determination required 15 days.

In the present study I have considered it would be of interest to investigate whether linearity exists for representatives of aldehyde derivatives (chloral hydrate), ureid derivatives (ectylurea and phenylcrotonylurea), phenothiazine derivatives (chlorpromazine). Haloperidol, which has previously shown an effect on spontaneous activity in small doses (cf. Chapter III), and serotonin, which as a biogeneous amine, should both be of interest in this connection too.

In the calculations, logarithmic values have been used at the reading time, usually 120 minutes, which gave clear response in the screening test in the manner described in the last chapter. Each determination has therefore required a number of days equal to the number of doses used, usually three days.

The determinations were carried out so that all the animals received all the doses at random.

In view of the results referred to in the last chapter, only one animal per cage was used in these determinations.

Chloral hydrate

The determination was carried out on 21 animals divided into three equal groups.

With the results given in the last chapter as a guide, the doses selected were 133, 200 and 300 mg/kg b.w. The substance was given in aqueous solution, each animal receiving 0.5 ml orally. The log 120-minute values were used for calculation.

Table 12 shows that the variance "departure from regression" is less than the experimental error and that the slope of the line departs from 0 ($P < 0.01$).

DEGERHOLM ET AL. (52) state that ED_{50} determined by the climbing test is 166 mg/kg b.w. for mice.

Ectylurea

Twenty-seven animals were used for this determination and were divided, as in the previous test, into three equal groups. The administered doses were 25, 50 and 100 mg/kg b.w. of the high-melting isomer and each animal receiving 0.5 ml orally. Here too, the log 120-minute values used were as the basis for calculation.

The variance of "departure from regression" exceeds the experimental error, but not by more than could be accounted for by chance ($P > 0.05$). The regression line showed a significant slope ($P < 0.001$).

KINNARD & CARR (11) have demonstrated using the photo-electric method that spontaneous activity decreased in mice given i.p. doses of between 10 and 50 mg/kg b.w. However, no statistical calculation had been made. In the same investigation they did not obtain any effect on co-ordination as determined with the rotating rod with i.p. doses of less than 375 mg/kg b.w.

Phenylcrotonylurea

It was described in the last chapter that the high-melting isomer of this substance gave a reduction in spontaneous activity down to 41 % of the placebo value. Therefore it would be of interest to study whether a linear dose-response curve could be obtained for oral doses of 50, 100 and 200 mg/kg b.w. The calculations were made on the log 60-minute values, since the other readings did not show any clear response with the two lower doses (cf. Chapter III).

The statistical analysis is given in Table 14.

The variance "departure from regression" is less than the error and the slope of the line is significant ($P < 0.02$).

Chlorpromazine

The selected representative of the tranquilizers of phenothiazine structure was chlorpromazine, which is often used as a control substance in studies of new substances of this type.

The doses used were 4, 6 and 9 mg/kg b.w. of the hydrochloride and were given in aqueous solution. The calculations were based on the log 120-minute values.

The departure from regression is less than the experimental error and the slope of the line is significant ($P < 0.001$).

As has already been mentioned, SHALLEK ET AL. (15) and SANDBERG (22) have given ED_{50} for white rats determined by the jiggle cage as 1.9 and 4.2 mg/kg b.w. respectively after subcutaneous administration. ED_{50} was defined as the dose which lowered spontaneous activity to 50 % of the control value. JANSEN ET AL. (83), using the rotating rod have given a value of 2.3 mg/kg b.w. after subcutaneous administration to mice.

Haloperidol

Since a very small dose of haloperidol have produced a reduction in spontaneous activity, I considered it would also be worthwhile to investigate whether a linear dose-response relationship existed for this substance. The doses used were 0.075, 0.15, 0.30 and 0.60 mg/kg b.w. The calculations were made on the log 120-minute values.

In the analysis of variance, the departure from regression was less than the experimental error but not greater than could be accounted for by chance ($P > 0.05$). The line showed a significant slope ($P < 0.001$). JANSEN ET AL. (83), using the rotating rod, have determined ED_{50} as 0.4 mg/kg b.w. subcutaneously.

Serotonin

Of the biogenic amines in the central nervous system, serotonin has been the subject of considerable interest. It might therefore be worthwhile to study this substance too. The doses were 1.25, 5 and 20 mg/kg b.w. (as creatinine sulphate). They were given i.p. in aqueous solution, as this substance is not very active when administered orally. The calculations were based on the log values two hours after administration.

The dose-response curve obtained did not depart from a linear regression. BROWN (24), in her investigations using the photo-electric method, obtained a response showing reduced spontaneous activity with i.p. doses of between 5 and 320 mg/kg b.w.; however, no calculations of the dose-relationship have been made.

Table 12. Test of linearity in the spontaneous activity dose-response curve.
Chloral hydrate

	<i>df</i>	Sum of squares	Mean square
Regression	1	3.7034	
Departure from regression.....	1	0.0382	0.0382
Between doses	2	3.7416	1.8708
Within	57	21.3871	0.3752
Total	59	25.1287	

Slope of regression line $b = -1.7280 \pm 0.5457$

$t_b = 3,167$

$P < 0,01$

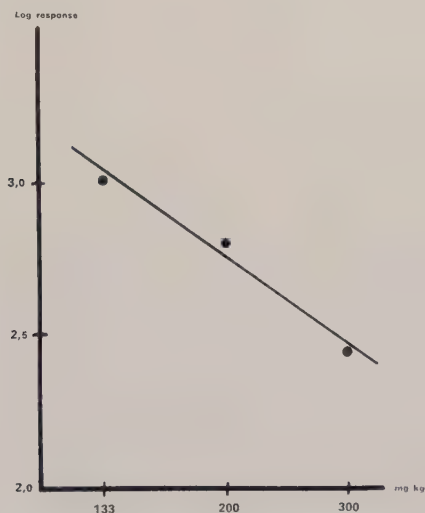


Fig. 36. The dose-response curve after orally administration of chloral hydrate to mice.

Table 13. Test of linearity in the spontaneous activity dose-response curve
Ectylurea

	<i>df</i>	Sum of squares	Mean square
Regression	1	10.6668	
Departure from regression.....	1	0.9127	0.9127
Between doses	2	11.5795	5.7898
Within	76	19.9169	0.2621
Total	78	31.4964	

$$F = \frac{0.9127}{0.2621} = 3.4823 \quad P > 0.5$$

Slope of regression line $b = -1.5053 \pm 0.2399$

$t_b = 6.279 \quad P < 0.001$

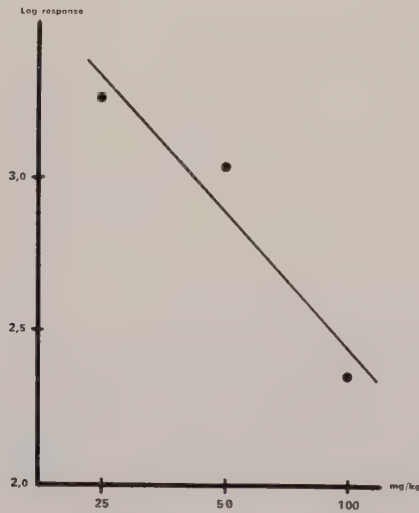


Fig. 37. The dose-response curve after orally administration of the high-melting isomer of ectylurea to mice.

Table 14. Test of linearity in the spontaneous activity dose-response curve
Phenylcrotonylurea

	<i>df</i>	Sum of squares	Mean square
Regression	1	1.8958	
Departure from regression	1	0.0061	0.0061
Between doses	2	1.9019	0.9510
Within	63	17.7256	0.2814
Total	65	19.6275	

Slope of regression line $b = -0.6897 \pm 0.2638$

$t_b = 2,616$

$P < 0.02$

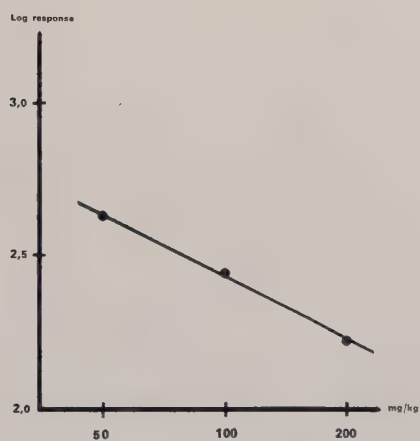


Fig. 38. The dose-response curve after orally administration of the high-melting isomer of phenylcrotonylurea to mice.

Table 15. Test of linearity in the spontaneous activity dose-response curve.
Chlorpromazine

	<i>df</i>	Sum of squares	Mean square
Regression	1	9.0050	
Departure from regression	1	0.1248	0.1248
Between doses	2	9.1298	4.5649
Within	66	11.6986	0.1773
Total	68	20.8284	

Slope of regression line $b = -2.5071 \pm 0.3509$

$t_b = 7.149$

$P < 0.001$

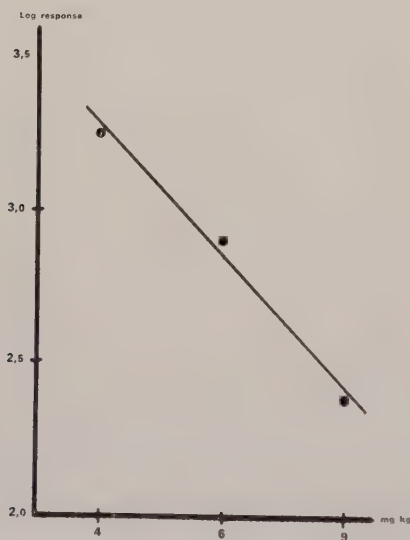


Fig. 39. The dose-response curve after orally administration of chlorpromazine chloride to mice.

Table 16. Test of linearity in the spontaneous activity dose-response curve.
Haloperidol

	<i>df</i>	Sum of squares	Mean square
Regression	1	8.2007	
Departure from regression	2	0.2592	0.1296
Between doses	3	8.4599	2.8200
Within	76	21.1977	0.2789
Total	79	29.6576	

Slope of regression line $b = -0.9515 \pm 0.1743$

$t_b = 5.460$

$P < 0.001$

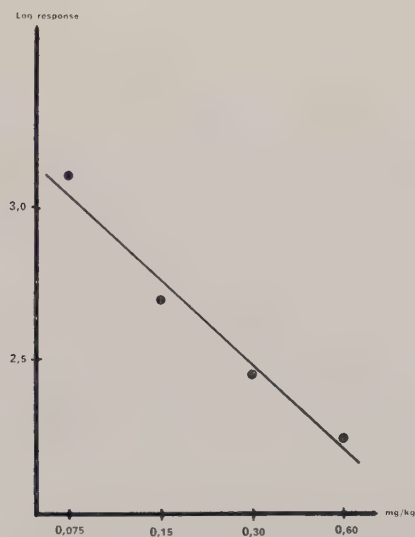


Fig. 40. The dose-response curve after orally administration of haloperidol to mice.

Table 17. Test of linearity in the spontaneous activity dose-response curve.
Serotonin creatinine sulphate

	<i>df</i>	Sum of squares	Mean square
Regression	1	2.7080	
Departure from regression	1	0.0524	0.0524
Between doses	2	2.7604	1.3802
Within	64	14.4777	0.2262
Total	66	17.2381	

Slope of regression line $b = -0.4133 \pm 0.1184$

$b = 3.482$ $P < 0.001$

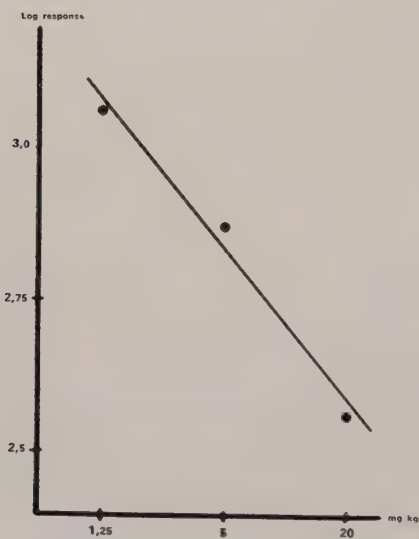


Fig. 41. The dose-response curve after intraperitoneally administration of serotonin creatinine sulphate to mice.

DISCUSSION

It should be possible to obtain a linear dose-response curve in a certain response range for any active drug with a measurable effect. The slope of this regression line may also be characteristic for the mode of action of the substance. A prerequisite for comparisons between different dosages is that the investigations are carried out under the most uniform possible conditions. The investigations described here therefore took the form of tests in which every animal received all the doses in random order. It would however be theoretically possible to administer a given dose to all the animals one day and a different dose to all of them on the following day over a five-day period, since it has not been possible to demonstrate any difference between days during five-hour periods on five consecutive days (cf. Chapter II). Thus if it is conceivable that a certain dose of a substance might continue to affect the spontaneous activity on the following day, this procedure can be adopted, *i.e.* the highest dose being given on the last day of the test. However, in all the investigations made in the present studies, a random order of the doses has been used.

All the substances have given a linear dose-response curve within the response range. Thus the linear dose-response curve for phenobarbital sodium obtained by LJUNGBERG (37) has proved, under the experimental conditions described here, to apply also to other groups of substances of known sedative effect, and it has been possible to reduce the test time to one day per dose, *i.e.* in most cases to three days.

There is thus reason to suppose that the test procedure used would be suitable for quantitative estimations of relative potencies of substances believed to possess similar sedative effects. Such studies have also been made and will be dealt with in the following chapter.

CHAPTER V

QUANTITATIVE STUDIES OF RELATIVE POTENCY

As described in the last chapter, linear dose-response curves were obtained for certain typical substances, indicating that the test procedure used could be applied to quantitative comparative studies of other substances believed to possess similar effects.

Therefore, substances such as are regarded as having the same effect or may be presumed by reason of their chemical structure to have it, were selected.

Thus chloral hydrate has been compared with dichloralphenazone, the high-melting isomer of ectylurea with the low-melting isomer, and chlorpromazine with perphenazine and haloperidol.

The substances used as controls have, as described in Chapter IV, all given a linear dose-response curve in a certain dose range.

Each animal was given 0.5 ml orally of an aqueous solution or a suspension of the preparation. The log values two hours after administration were used as the basis for the statistical calculations. The doses were selected so that the values would fall on the linear part of the log dose response curve.

Chloral hydrate and dichloralphenazone

Doses: Chloral hydrate C_1	= 200 mg/kg b.w.
C_2	= 300 mg/kg b.w.
Dichloralphenazone D_1	= 313 mg/kg b.w.
D_2	= 470 mg/kg b.w.

Since dichloralphenazone is a compound of two molecules of chloral hydrate and one molecule of phenazone, the doses of dichloralphenazone were adjusted to correspond to 200 and 300 mg/kg b.w. respectively of chloral hydrate.

The results show that the effect of the amount of dichloralphenazone administered corresponded to 102 % of that of chloral hydrate. There was a significant slope ($P < 0.001$) and no departure from parallelism could be established ($P > 0.05$). A measure of the precision of assay is given according to GADDUM (48) by the ratio between the standard deviation (s)

Table 18. Factorial analysis of effect on spontaneous activity in mice.
Relation between chloral hydrate (C) and dichloralphenazon (D)

	df	Sum of squares	Mean square	Variance ratio	P
Between doses.....	3	10.8304	3.6101	7.11	< 0.01
Between animals.....	27	28.8277	1.0677	2.10	< 0.01
Error.....	81	41.1389	0.5079	—	
Total	111	80.7970			

	Factorial coefficients (x)				Divi- sor	Sum of products	Mean square	Variance ratio	P
	C ₁	C ₂	D ₁	D ₂					
Diff. between prep. .	— 1	— 1	+ 1	+ 1	112	— 1.6017	0.0229	22.18	>0.05
Slope	— 1	+ 1	— 1	+ 1	112	— 34.7025	10.7524	21.15	<0.01
Departure from parallelism	+ 1	— 1	— 1	+ 1	112	— 2.4855	0.0552	9.24	>0.05
Total dose response	74.9728	58.8643	75.4147	56.8207					

$$i = 0.1761$$

$$b = 3.5190$$

$$\lambda = \frac{s}{b} = 0.20$$

Relative potency 101.9 % (Fiducial limits at the 0.05 probability level
70.3—142.3 %)

and the slope (*b*). This ratio is represented by the symbol λ and was found to be 0.20. (Table 18).

In a comparative study with reference to HD_{50} , dichloralphenazone had an effect which was 61.8 % (54.8 %—68.7 %, $P = 0.05$) of that of chloral hydrate; this corresponds closely to the chloral hydrate content of dichloral phenazone, which is 64 %.

A comparative toxicity test revealed a relative toxicity of 69.9 % (64.2 %—76.1 %, $P = 0.05$), which also corresponds fairly well to the chloral hydrate contents.

Since both the effect on spontaneous activity and the HD_{50} of dichloralphenazone could be wholly attributed to its chloral hydrate contents it would be interesting to study the durations of hypnosis when equivalent amounts of chloral hydrate were given. The results are given in Table 19, which shows that no difference between the two preparations could be established in this respect either.

Table 19. Duration of dichloralphenazone and chloral hydrate in mice

	Route	Dose mg/kg	Duration of hypnosis min. $\pm$ S.E.
Dichloralphenazone	i. p.	470	23.5 $\pm$ 2.54
	i. v.	390	24.0 $\pm$ 3.30
Chloral hydrate	i. p.	300	25.2 $\pm$ 2.02
	i. v.	250	21.8 $\pm$ 3.03

Ectylurea, high- and low-melting isomers

Doses: Ectylurea isomer $H_1 = 25$ mg/kg b.w.

m.p. 193°C $H_2 = 50$ mg/kg b.w.

Ectylurea isomer $L_1 = 25$ mg/kg b.w.

m.p. 158°C $L_2 = 50$ mg/kg b.w.

There was a significant difference ($P < 0.001$) between the preparations with respect to their influence on spontaneous activity. (Table 20).

Since the dose-response curves are not parallel, no direct comparison of their relative potency can be made.

Calculations were able to establish a significant slope for the high-melting isomer only, so that the low-melting isomer does not seem to possess any effect at all in this dose range. (Table 21).

Table 20. Factorial analysis of effect on spontaneous activity in mice.
Relation between the high (H) and low (L) melting isomers of ectylurea

	df	Sum of square	Mean square	Variance ratio	P
Between doses	3	6.8463	2.2821	15.21	< 0.01
Between animals	24	13.3334	0.5556	3.70	< 0.01
Error	72	10.8113	0.1502	—	
Total	99	30.9910			

	Factorial coefficients (x)				Divisor	Sum of products	Mean square	Variance ratio	P
	H_1	H_2	L_1	L_2					
Diff. between prep. .	— 1	— 1	+ 1	+ 1	100	—18.0372	3.2534	21.66	< 0.01
Slope	— 1	+ 1	— 1	+ 1	100	—15.6330	2.4439	16.27	< 0.01
Divergence in slope .	+ 1	— 1	— 1	+ 1	100	10.7476	1.1551	7.69	< 0.01
Total dose response	79.5575	66.3672	83.2023	80.7596					

Table 21. Slope of log dose-response curves of the isomers of ectylurea

	Slope	<i>t</i>	<i>df</i>	<i>P</i>
Ectylurea m.p. 193° C.	0.584 ± 0.171	3.42	49	< 0.01
Ectylurea m.p. 158° C.	0.098 ± 0.099	0.986	49	0.4—0.3

Chlorpromazine and perphenazine

Doses: Chlorpromazine $Ch_1 = 4$ mg/kg b.w.

$Ch_2 = 8$ mg/kg b.w.

Perphenazine $P_1 = 1$ mg/kg b.w.

$P_2 = 2$ mg/kg b.w.

It would be of interest to investigate the possibility of making a quantitative comparison between chlorpromazine and perphenazine. (Table 22).

Perphenazine required only a dose of about 25 % of that of chlorpromazine to produce the same reduction in spontaneous activity. There was no departure from parallelism but a significant slope ($P < 0.01$). SANDBERG (22) gives ED_{50} for chlorpromazine as determined by the jiggle cage as 4.2 mg/kg b.w. and for perphenazine as 0.2 mg/kg b.w. administered s.c. to white rats; JANSEN ET AL. (83), using the rotating rod, give the respective s.c. figures as 2.3 and 0.46 mg/kg b.w. for mice, but no comparative statistics have been worked out.

Chlorpromazine and haloperidol

Doses: Chlorpromazine $Cp_1 = 4$ mg/kg b.w.

$Cp_2 = 8$ mg/kg b.w.

Haloperidol $Hp_1 = 0.2$ mg/kg b.w.

$Hp_2 = 0.4$ mg/kg b.w.

JANSEN ET AL. (83) report that haloperidol has an effect which is qualitatively similar to but quantitatively greater than that of chlorpromazine. CARLSSON & LINDQUIST (114) have also demonstrated that although the two substances have a different chemical structure they exerted a similar effect on the metabolism of brain catecholamines. The investigations described in Chapter III might also possibly be taken to indicate similar mode of action. To obtain a direct quantitative comparison, a four-point assay was made with the doses quoted above.

Thus it only required about one-twentieth as much haloperidol as chlorpromazine to produce the same effect on spontaneous activity.

Table 22. Factorial analysis of effect on spontaneous activity in mice.
Relation between chlorpromazine (Ch) and perphenazine (P)

	df	Sum of squares	Mean square	Variance ratio	P
Between doses.....	3	5.1741	1.7247	8.16	< 0.01
Between animals.....	21	10.4810	0.4991	2.36	< 0.01
Error.....	63	13.3170	0.2114		
Total	87	28.9721	0.4598		

	Factorial coefficients (x)				Divi- sor	Sum of products	Mean square	Variance ratio	P
	Ch ₁	Ch ₂	P ₁	P ₂					
Diff. between prep. .	— 1	— 1	+ 1	+ 1	88	1.7207	0.0336	6.29	> 0.05
Slope	— 1	+ 1	— 1	+ 1	88	—19.9497	4.5230	21.40	< 0.01
Departure from parallelism	+ 1	— 1	— 1	+ 1	88	7.3791	0.6188	2.93	> 0.05
Total dose response.	68.2543	54.5899	65.4251	59.1398					

$$i = 0.3010$$

$$b = 1.5063$$

$$\lambda = \frac{s}{b} = 0.30$$

Relative potency 94.2 % (Fiducial limits at the 0,05 probability level 74.0—135.2 %)

Table 23. Factorial analysis of effects on spontaneous activity in mice.
Relation between chlorpromazine (Ch) and haloperidol (Hp)

	df	Sum of squares	Mean square	Variance ratio	P
Between doses.....	3	1.0123	0.3374	3.34	0.05—0.01
Between animals.....	22	3.4401	0.1564	1.55	0.05—0.01
Error.....	66	6.6700	0.1011		
Total	91	11.1223	0.3179		

	Factorial coefficients (x)				Divi- sor	Sum of products	Mean square	Variance ratio	P
	Ch ₁	Ch ₂	Hp ₁	Hp ₂					
Diff. between prep. .	— 1	— 1	+ 1	+ 1	92	1.7796	0.0344	2.94	> 0.05
Slope	— 1	+ 1	— 1	+ 1	92	—9.4666	0.9741	9.64	< 0.01
Departure from parallelism	+ 1	— 1	— 1	+ 1	92	0.5867	0.0037	27.02	> 0.05
Total dose response.	75.5653	70.5386	76.1617	71.7217					

$$i = 0.3010$$

$$b = 0.6836$$

$$\lambda = \frac{s}{b} = 0.47$$

Relative potency 87.8 % (Fiducial limits at the 0,05 probability level 63,1—158,4 %)

DISCUSSION

Statistical methods can be used not only to estimate the true effect but also to provide the means for a quantitative evaluation of the effects of various drugs. The experiments mentioned in this chapter show that it is possible to make an assessment of substances with assumed similar effect on spontaneous activity.

As to the comparison between chloral hydrate and dichloralphenazone it is of interest to note that the activity of dichloralphenazone can be entirely attributed to the contents of chloral hydrate, despite the fact that phenazone in doses of 300 mg/kg b.w. previously brought about a reduction in spontaneous activity (Chapter III). However, the lower dose of 100 mg/kg b.w. had no effect. The doses of phenazone in the dichloralphenazone were 113 and 170 mg/kg b.w. respectively. The fact that the dose-effect lines of the two preparations do not diverge does not mean that the preparation, as far as these properties are concerned, have the same modes of action, but only that this possibility exists.

As to the two isomers of ectylurea, it is of interest to note that there is a significant difference in the effect on spontaneous activity at the dose levels tested. The dose-effect curve of the isomer with the lower-melting point did not have a significant slope. On the other hand, the isomer with the higher-melting point had such, which indicates that this isomer at these dose levels does have a pharmacological effect on spontaneous activity. In earlier experiments both on rats (69) and mice (70) the low-melting isomer had a toxicity twice as great.

Perphenazine is claimed to be as effective therapeutically as chlorpromazine, but in smaller doses (115, 116). In these experiments perphenazine brought about an activity which was about four times as great as that of chlorpromazine. JANSEN ET AL. (83), using the rotating rod obtained similar values with mice, while SANDBERG (22) with the jiggle cage obtained the same effect on white rats with perphenazine with doses which were only one-twentieth of the doses required to attain the same effect with chlorpromazine, and climbing test required about half the dose.

In my experiments haloperidol has given the same effect as chlorpromazine with only one-twentieth of the chlorpromazine dose. Consequently, the results correspond well with those described in Chapters III and IV.

If the values obtained by JANSEN ET AL. (83) with chlorpromazine and haloperidol are compared, five times as large a dose with chlorpromazine is required to attain the same effect with the rotating rod as with haloperidol. The test method which I have used gives a quantitatively larger difference.

The results show that it is evidently possible with the test method used to make quantitative assessments of preparations with assumed similar effect on spontaneous activity, as well as making it possible, in certain cases, to reveal differences in the modes of action of the substances by evaluating the parallelism. An example of the latter is provided by the experiments on the two isomers of ectylurea.

Substances which in the screening test previously described have proved to be of interest can thus be compared to known preparations with assumed similar effect. Four days were required for each assessment.

CHAPTER VI

GENERAL DISCUSSION

Numerous different methods have been used in experiments on animals for the study of sedative effect of various drugs. No completely satisfactory method has been found as yet, which probably is primarily due to the physiology and biochemistry of the brain still being unknown to a great extent. Moreover, there is the difference between various animals and of the metabolism of different kinds of animals concerning the substances administered. In order to form an opinion about a centrally active substance it is thus necessary to use several different methods — especially biochemical, physiological and pharmacological — and also to carry out the experiments on different types of animals.

In recent years the use of statistical calculations has created quite different opportunities of assessing various methods. By calculating the relationship between dose and effect, better data about the drug can be obtained than with qualitative studies. Often a quantitative study can give a certain idea about the mode of action of the drug in relation to other pharmaceuticals.

When deciding upon apparatus for screening and follow-up studies, I thought it necessary to examine an apparatus which earlier had given a linear dose-effect curve for a drug with known sedative effect.

One of the reasons why I wished to study this apparatus more closely was that spontaneous activity could be measured by it and that a reduction in spontaneous activity is one of the most obvious signs of sedation in animals. Other reasons were that this test method gives an objective measurement of the effects obtained and that the test animals could be mice, as both screening and possible follow-up studies require large number of animals. Another reason for my choice was that with this apparatus the animals can live in conditions resembling the normal and can continue their nocturnal activity without being stimulated by other drugs or being affected in any other way. On account of its design the apparatus also gives a certain idea of the initiative of the animals and thus also of their behaviour pattern.

If the straight-line dose-effect curve obtained by LJUNGBERG (37) for phenobarbital sodium can also be obtained for other drugs with sedative effect, it should be possible to carry out comparative follow-up studies on a series of chemically similar substances with presumed similar pharmacological effect.

However, the method used by LJUNGBERG is far too time-consuming for the study of a series of substances. I have therefore endeavoured to simplify the test procedure both for screening and for follow-up studies.

The results obtained must consequently be regarded in relation to both the normal variation in the test procedure and to the doses administered, as each substance in a dose which produces a toxic effect probably can cause a reduced spontaneous activity with this apparatus.

For measuring the variation I have used the relative standard deviation ($s_{\%}$) after the animals had adjusted their daily rhythm in such a way that their active period (nocturnal activity) coincided with the working hours of the laboratory. This adjustment took about ten days (Figure 4) and during this time the animals also learned to use the apparatus and adjust themselves to their new environment. A special experiment showed that the time required for training was about six days. After the pre-period the low variance of the spontaneous activity from day to day was striking.

As the animals, when placebo was administered, did not show any greater variation either in the number of revolutions or in standard deviation during the five first hours of the nocturnal activity, the test period could be prolonged over this period. DEWS (51) states that when using the photo-electric method the variation increases successively and is smallest during the first 15-minute period; the same can be seen from the result obtained with the same method by BROWN ET AL. (14) WINTER & FLATAKER (26) and MELANDER (27) also used short test periods with the photo-electric method. SANDBERG (22), on the other hand, in his experiments with white rats and jiggle cage extended the test period over the entire dark period in his studies of various phenothiazine derivatives.

In studies of spontaneous activity it is not uncommon to place several animals in the same cage (14, 24, 25, 27, 37, 51) in order to obtain smaller variations. BROWN (107) considers also that "... doses of the classical hypnotic agents which depress isolated spontaneous activity of grouped mice are many times those required to depress isolated mice, whereas doses of the new non-hypnotic depressant which suppress groups' spontaneous activity and which depress individual activity are similar".

LJUNGBERG used two mice in each cage in his experiments, but I consider it to be of interest also to use one mouse per cage with this test method. Here it appeared that no definite difference in variation ($s_{\%}$) was obtained.

However, the administration of various drugs with one animal per cage produced almost throughout a greater reduction in spontaneous activity than with two animals per cage and this method was thus to be preferred.

Here there was no tendency similar to that found by BROWN (107),

between one and two animals per cage. In the preliminary experiments with stimulating agents it is, however, interesting to note that with the same dose of amphetamine quite different results were obtained when one or four animals were used in the experiments. It should be fairly easy to design an apparatus which is a combination of the apparatus used here and the photo-electric method by placing photo-electric cells close to the bottom of the cage. In this way such an apparatus could give a certain idea of the behaviour-pattern of the animals. Also for other substances producing excitement with loss of initiative such an apparatus could be valuable for the study of possible effects.

As has been emphasized before, the studies of known drugs were made in order to be able to assess the results and to relate them to results obtained in experiments with new substances. If the previously determined double relative standard deviation is set as the limit for what is of interest or not, it appeared that hypnotics and sedatives required relatively large doses in comparison with LD_{50} . A dose between 10 % and 20 % of LD_{50} would probably be necessary to ensure that no substances with assumed similar effect would be lost. Such a dosage would also be required for glutethimide, meprobamate, imipramine, iproniazid, promethazine, phenbenzamine, acetylsalicylic acid and phenazone. Iproniazid, phenbenzamine and acetylsalicylic acid produced effects which barely exceed this limit. These substances would hardly have straight-line dose-effect curves. In order to attain greater effect on spontaneous activity one would probably have to give toxic doses. The sedative effect of both phenbenzamine and acetylsalicylic acid is also considered to be very slight and is entirely lacking for iproniazid. BENSON & SCHIELE (111) considers that imipramine as well as other phenothiazine derivatives have a sedative effect, and results with a centrally depressing effect, such as attained in these experiments, have been obtained by DOMENJOZ & THEOBALD (92) and SIGG (93).

The fact that both meprobamate and promethazine gave results at the dose level, which in relation to LD_{50} can be compared with that of hypnotics and sedatives, corresponds well with other experiments on animals (9, 15, 82, 83).

It is true that these rather high doses in relation to LD_{50} may produce an acute toxic effect. However, the substances tested here have in no instance produced such an effect. A sort of guarantee for this is that after the maximum effect the animals generally resume their normal spontaneous activity even during the test period.

DEWS (51) in his work on stimulating agents using the photo-electric method recommend the use of doses of 10 % and 50 % of LD_{50} for screening.

Substances which require up to 50 % of LD_{50} to produce an effect with this test method are probably not of interest as sedatives, as the slope of the toxic dose-effect curve is rarely so steep that half the LD_{50} dose would not have toxic effects.

Morphine has brought about a reduction in spontaneous activity with doses which in relation to LD_{50} are considerably smaller than those which are generally required with substances belonging to the group sedatives and hypnotics.

Ectylurea and thalidomide gave effects with doses which in relation to LD_{50} corresponded more closely to those produced with major tranquilizers, namely approx. 1 %—2 % of LD_{50} . These results also correspond with those obtained from previous experiments on animals (11, 21, 68, 69, 70).

Major tranquilizers as reserpine, chlorpromazine and haloperidol only needed doses of 1 % of LD_{50} in order to cause a reduction of more than twice the standard deviation. Thus the relation to LD_{50} of these substances is extremely favourable and this corresponds well with the results of previous experiments using these substances (3, 13, 15, 22, 82, 83).

Emylcamate gave quite different results than meprobamate; instead of a reduction of spontaneous activity emylcamate caused stimulation during the first hour. These results differ completely from those obtained using the photo-electric method. The results here can almost be compared with those obtained in the psychotechnical experiments of JONSON & ANDERSEN (110). In the latter experiments there was no lowering of capability even with doses as high as 1 200 mg.

The completely different results obtained from the two isomers of phenylcrotonylurea are of interest. The low-melting isomer produced a stimulation at the same dose level where the high-melting isomer caused a reduction, i.e. parallel results as those obtained previously with the two isomers of ectylurea (70, 71).

In the present experiment a study has been made of potentiated narcosis at the same dose level as that used in the studies of spontaneous activity. There was no potentiated narcosis with haloperidol, reserpine and morphine, despite the fact that these drugs caused a marked reduction in spontaneous activity. Nor emylcamate, phenbenzamine, the low-melting isomer of phenylcrotonylurea, caffein and amphetamine produced potentiated narcosis.

The results obtained with caffein are difficult to interpret, but may possibly be due to the loss of ability to take initiative or to co-ordinate movements. According to STERNER (117) doses close to LD_{50} are required to cause activity pattern in the electroencephalogram of rabbits.

Assuming that the reduction in spontaneous activity is to be regarded as a "pure" pharmacological effect, it should be possible to obtain a linear dose-effect curve for preparations giving this effect. Representative substances from the different groups have shown that this was the case with the dose level used in the screening test. This also opens up the possibilities of carrying out comparative quantitative studies of substances with presumed similar effect.

Due to the fact that it has been possible to establish the linearity of different groups, it should be sufficient to use four-point assay instead of six-point assay, and consequently shortening the time needed for the experiment.

In a comparative study between chloral hydrate and dichloralphenazone no difference in parallelism could be determined and consequently there is no criterion for the different modes of action of the two drugs with the method used here. The fact that LD_{50} , HD_{50} and sleeping time have given corresponding results in equimolecular amounts of chloral hydrate verifies the possibility that the squirrel wheel method had measured the pure "sedative" effects of both these drugs.

In the quantitative experiments, neither was there any deviation from parallelism when chlorpromazine was compared with perphenazine and haloperidol. This gives the possibility that they could have the same mode of action, which would be especially interesting in the case of haloperidol, where the difference in chemical structure is more marked. CARLSON & LINDQUIST (114) have studied the effects of chlorpromazine and haloperidol on mice and have found a possibly similar modes of action on the metabolism of brain catecholamines.

On the other hand, where the ectylureas were compared both isomers showed a difference in parallelism. Probably this difference could be attributed to the low-melting isomer, as the slope does not deviate from 0. Thus, if an inactive substance in an earlier screening test has wrongly been found "of interest" the following quantitative test can give the possibility to correct this mistake.

No completely satisfactory method has been found by which — in the clinical sense — the sedative effect of a drug can be determined in experiments on animals. In order to undertake experimental pharmacological studies of drugs having such effects, a combination of several methods is required. I have found it to be of interest to go more thoroughly into one of the existing possibilities, especially with regard to the opportu-

ity of following up those substances, which after screening prove to be of interest. Here it is important to give quantitative statistical aspects to the dose-effect relationship, where the straight-line curve, slope and parallelism come to the fore so that the pharmacological effects can be judged with accuracy.

SUMMARY

Introduction. — No completely satisfactory method has been found to study the sedative effects of drugs in experiments on animals. The reason for this may probably be the difficulty in delimiting the measurable effects on animals from other effects which can be said to be sedative and which could influence the results. In order to determine the sedative effect on animals it is therefore necessary to use several different methods. These should be well tested both for the normal variation of the method and for administration of known drugs.

The object of the present investigation is the closer study of a modified method of the squirrel wheel technique, in order to find out the suitability of this apparatus for screening and for quantitative follow-up studies of substances with presumed central depressive effect.

The reason why I have chosen this apparatus is pointed out.

Chapter I. — Standard *statistical methods* have been used and $P = 0.05$ was taken as the limit of the probability level.

Experimental animals. Male albino mice weighing between 18 and 23 g from the same animal supplier have been used for determining the spontaneous activity, the median lethal dose (LD_{50}), the median hypnotic dose (HD_{50}) and potentiated narcosis. Altogether about 6 000 mice have been used.

Apparatus used for determination of spontaneous activity. — The modified squirrel wheel method used differs from the most commonly used squirrel wheel in that the animals do not necessarily have to be inside the wheel but have the possibility of jumping into or out of the wheel voluntarily. Different experiments were carried out having one or two animals in each cage.

The revolutions were registered electrically with two comptometers for each wheel and altogether about 70 000 readings have been taken.

Administration of drugs. — In all the experiments, with exception of the dose-effect studies on serotonin and hexobarbital sodium in potentiated narcosis, which were given intraperitoneally, the preparations were given per os.

Determination of the median lethal dose (LD_{50}) and median hypnotic dose (HD_{50}) was calculated using probit analysis and at least 30 mice were used for each calculation.

Chapter II. — The studies of spontaneous activity took place during nocturnal activity, which is the active period for mice. The importance of

training and adaption is pointed out. In determining the standard deviation for the mean values from day to day over a 15-day period this was found to be satisfactory low both with one and two animals per cage. No difference between the relative standard deviations could be noted. The spontaneous activity as well as the relative standard deviation did not vary noticeably either with one or two animals per cage during a five-hour period. The relative standard deviation for diurnal activity as a rule was more than three times greater than during nocturnal activity. Consequently, experiments with substances which cause a lowering of spontaneous activity should preferably not be carried out during diurnal activity.

Chapter III. — The suitability of the apparatus for screening substances with assumed sedative effect has been studied. In order to be able to evaluate the test method and put the results obtained in relation to these obtained with new substances with assumed sedative effect, some 30 known drugs, primarily those with sedative effect, have been studied. All substances with a proved sedative effect gave a reduction in spontaneous activity which was more than twice the standard deviation where the substances were administered in doses of up to 20 % of LD₅₀. The fact that also other substances produce this effect is shown and discussed.

The test doses of the different preparations have been put in relation to their toxicity and hypnotic effect. The choice of dose and the results attained are discussed and compared with those obtained with other methods and with the potentiated narcosis at the dose level determined here.

A reading time over a period of two hours seems in the main to be sufficient, but as the test period can be extended over five hours, continuous readings during the entire test period should give a certain idea of the induction time and duration of the substances.

Chapter IV. — It is shown that different types of substances within a certain range of effect give a linear dose-effect curve. Consequently, within certain dose intervals a straight-line dose-effect curve is given by all the following: chloral hydrate, ectylurea, phenylcrotonylurea, chlorpromazine, haloperidol and serotonin. For each evaluation as many days were needed as the number of doses used in the experiment, generally three days.

Chapter V. — The experiments show that an evaluation of substances with assumed similar effect on spontaneous activity can be made with this test method by means of four point assays. Slope and parallelism were statistically evaluated and the index of precision of the factorial analyses were sufficient.

Chapter VI. — In order to undertake experimental pharmacological studies of drugs having a sedative effect, a combination of several methods is required. In this investigation one of the existing possibilities has been the subject of more thorough studies, especially with regard to the opportunity of following up those substances which after screening prove to be of interest. Here it is important to give, by means of statistical analysis, the quantitative aspects to the dose-effect relationship so that the pharmacological effect can be judged with accuracy.

REFERENCES

1. RILEY H. and SPINKS A., *J. Pharm. Pharmacol.* 10, 657 (1958) and 10, 721 (1958).
2. WINTER, C., *J. Pharmacol. Exp. Ther.* 94, 7 (1948).
3. LESSIN, A. and PARKES N., *Brit. J. Pharmacol.* 12, 245 (1957).
4. LESSIN, A. and PARKES N., *J. Pharm. Pharmacol.* 9, 657 (1957).
5. BIANCHI, C., *Arch. int. Pharmacodyn.* 3, 227 (1957).
6. KOPERA, J. and ARMITAGE, A., *Brit. J. Pharmacol.* 9, 392 (1954).
7. THOMPSON, R., *Endocrinology* 39, 62 (1946).
8. NIESCHULZ, O., POPENDIKER, K. and HOFFMAN I., *Arzneimittelforschung* 5, 680 (1955).
9. TRIPOD, J. and GROSS F., *Helv. physiol. Acta* 15, 105 (1957).
10. DUNHAM, N. and MIYA, T., *J. Amer. pharm. Ass., sci. Ed.* 46, 208 (1957).
11. KINNARD, W. and CARR, C., *J. Pharmacol. Exp. Ther.* 121, 354 (1957).
12. KNEIP, P., *Arch. int. Pharmacodyn.* 126, 238 (1960).
13. COOK, L., WEIDLY, E., MORRIS, R. and MATTIS, P., *J. Pharmacol. Exp. Ther.* 113, 11 (1955).
14. BROWN, B., BRAUN, D. and FELDMAN R., *J. Pharmacol. Exp. Ther.* 118, 153 (1956).
15. SHALLEK W., KUEHN, A. and SEPPELIN, K., *J. Pharmacol. Exp. Ther.* 118, 139 (1956).
16. BERGER, F., *J. Pharmacol. Exp. Ther.* 112, 413 (1954).
17. SZYMANSKI, I., *Pflueger Arch. Ges. Physiol.* 158, 343 (1914).
18. ASCHOFF, J., *Pflueger Arch. Ges. Physiol.* 254, 262 (1951).
19. SCHLAGINTWEIT, E., *Arch. exp. Path. Pharmacol.* 131, 212 (1928).
20. IRRGANG, K., *Fort. Ther.* 18, 145 (1942).
21. KUNZ, W., KELLER, H. and MÜCKTER, H., *Arzneimittelforschung* 6, 426 (1956).
22. SANDBERG, F., *Arzneimittelforschung* 9, 203 (1959).
23. SIEGEL, P., *J. Psychol.* 21, 227 (1946).
24. BROWN, B., *Ann. N. Y. Acad. Sci.* 66, 677 (1957).
25. FRIEBEL, H., SOMMER, S. and VARADAN, K., *Arzneimittelforschung* 9, 126 (1959).
26. WINTER, C. and FLATAKER, L., *J. Pharmacol. Exp. Ther.* 103, 93 (1951).
27. MELÄNDER, B., *J. Med. Pharm. Chem.* 1, 443 (1959).
28. STEWART, C., *Amer. J. Physiol.* 1, 40 (1898).
29. SLONAKER, J., *Anat. Rec.* 2, 116 (1908).
30. HEMMINGSEN, A. and KRARUP, N., *Biol. Medd. Kbh. suppl.* 7, 13 (1937).
31. PARK, O. and WOODS, L., *Proc. Soc. Exp. Biol.* 43, 366 (1940).
32. RICHTER, C. and WANG, G., *J. Lab. Clin. Med.* 12, 289 (1926).
33. SEARLE, L. and BROWN, C., *J. Exp. Psychol.* 22, 480 (1938).
34. KOCH, F., *Zschr. exper. Med.* 106, 445 (1939).
35. JONES, D., KIMELDORF, D., RUBADEAU, D. und CASTANERA, T., *Amer. J. Physiol.* 172, 109 (1953).
36. EISENHUTH, F., EULNER, H. and KAISER, W., *Wiss. Z. Univ. Halle, Math.-Nat.* 4, 423 (1955).
37. LJUNGBERG, S., *Acta Pharmacol. (Kobenhavn)* 13, 96 (1957).
38. SHIRLEY, M., *J. Comp. Psychol.* 8, 23 (1928).
39. LACEY, O., *Amer. J. Psychol.* 57, 412 (1944).
40. FORST, A., *Arch. exp. Path. Pharmacol.* 189, 288 (1938).
41. SIEGMUND, P. and WOLF, M., *Arch. exp. Path. Pharmacol.* 216, 323, (1952).
42. FORST, A., *Arch. exp. Path. Pharmacol.* 192, 257 (1939).

43. LAURENCE, D., *Prescribers J.* 2, 38, (1962).
44. BLISS, C., *The Statistics of Bioassay*, New York (1952).
45. BURN, J., *Biological Standardization*, London (1950).
46. FINNEY, D., *Statistical Method in Biological Assay*, London (1952).
47. FISHER, R. and YATES, F., *Statistical Tables for Biological, Agricultural and Medical Research*, London (1953).
48. GADDUM, J., *Pharmacol. Rev.* 5, 87 (1953).
49. LJUNGBERG, S., *Kompendium i statistik* (1960).
50. *Pharmacopoea Nordica Vol. IV*, Stockholm (1960).
51. DEWS, P., *Brit. J. Pharmacol.* 8, 46, (1953).
52. DEGERHOLM, E., HANSSON, A., LEIDEMAN, T. and STERNER, N., *Farm. Revy* 62, 601, (1962).
53. REINHARD, J., KIMURA, E. and SCUDI, J., *J. Pharmacol. Exp. Ther.* 106, 444 (1952).
54. RICE, W. and MCCOLL, J., *J. Amer. Pharm. Ass. sci. Ed.* 14, 137 (1956).
55. EEROLA, R., *Ann. Med. Exp. Biol. Fenn. suppl.* 13, 39 (1961).
56. SANDBERG, F., *Acta Physiol. Scand.* 22, 311 (1951).
57. FEARN, J. and HODGES, J., *J. Pharm. Pharmacol.* 5, 1041 (1953).
58. RAMSEY, H. and HAAG, H., *J. Pharmacol. Exp. Ther.* 88, 313 (1946).
59. MARGOLIN, S., PERLMAN, P., VILANNI, F. and MCGAVACK, *Science* 114, 384 (1951).
60. BARNES, J., MCCREA, P., MARSHALL, P., SHEAHAN, M. and WALSH P., *J. Pharm. Pharmacol.* 10, 315 (1958).
61. MARLEY, E., *Brit. J. Pharmacol.* 14, 284 (1959).
62. GROSS, F., TRIPOD, J. and MEIER, R., *Schweiz. Med. Wschr.* 85, 305 (1955).
63. COOK, L. and WEIDL, E., *Ann. N. Y. Acad. Sci.* 66, 740 (1957).
64. v. D. EECKHOUT, A., *Arch. exp. Path. Pharmacol.* 57, 338 (1907).
65. KRIEGER, H. and v. D. VELDEN, R., *Deutsch. Med. Wschr.* 33, 213 (1907).
66. IMPENS, E., *Med. Klinik* 6, 1861 (1910).
67. FLEISCHMANN, P., *Med. Klinik* 6, 1860 (1910).
68. FINCKH, J., *Med. Klinik* 6, 1860 (1910).
69. PINDELL, M., FANCHER, O. and LIM, R., *Fed. Proc.* 12, 357 (1953).
70. LJUNGBERG, S. and STRÖM, S., *Svensk Farm. T.* 62, 121 (1958).
71. FANCHER, O. and LIM, R., *Arch. Int. Pharmacodyn.* 114, 418 (1958).
72. FERGUSON, J. and LINN, R., *Antibiot. Med. Clin. Ther.* 3, 329 (1956).
73. FERGUSON, J., *J. Am. Ger. Soc.* 4, 1080 (1956).
74. WEAVER, J. and MIYA, T., *J. Pharm. Sci.* 50, 910 (1961).
75. WEESE, H. and SHARPF W., *Deutsch. Med. Wschr.* 58, 1205 (1932).
76. SHONLE, H. and MOMENT, A., *J. Amer. Chem. Soc.* 45, 243 (1923).
77. SWANSON, E. and PAGE, J., *J. Pharmacol. Exp. Ther.* 31, 1 (1927).
78. DOX, A. and HJORT, A., *J. Pharmacol. Exp. Ther.* 31, 455 (1927).
79. IMPENS, E., *Deutsch. Med. Wschr.* 38, 945 (1912).
80. LOEWE, S., *Deutsch. Med. Wschr.* 38, 947 (1912).
81. JULIUSBURGER, O., *Berl. Klin. Wochenschr.* 49, 940 (1912).
82. CAVIEZEL, R. and BAILLOD, A., *Pharm. Acta Helv.* 33, 469 (1958).
83. JANSSEN, P., VAN DE WESTERINCH, C., JAGENEAU, A., DEMOEN, P., HERMANS, B., VAN DAELE G., SCHELLEKENS, K., VAN DER EYCKEN, C. and NIEMEGERES, C., *J. Med. Pharm. Chem.* 1, 281 (1959).
84. TAGMANN, E., SURY, E. and HOFFMAN, K., *Helv. Chim. Acta* 35, 1235 (1952).
85. MÜLLER, P. and ROHRER, F., *Schweiz. Med. Wschr.* 85, 309 (1955).
86. STRÖM, S., to be published.

87. BEIN, H., GROSS, F., TRIPOD, J. and MEIER, R., *Schweiz. Med. Wschr.* **83**, 1007 (1953).
88. PLUMMER, A., EARL, J., SCHNEIDER, J., TRAPOLD, J. and BARRET, W., *Ann. N. Y. Acad. Sci.* **59**, 8 (1954).
89. COURVOISIER, S., FOURNEL, J., DUCROT, R., KOLSKY, M. and KOETSCHET, P., *Arch. Int. Pharmacodyn.* **92**, 305 (1953).
90. LJUNGBERG, S., *J. Pharm. Belg.* **41**, 115 (1959).
91. SCHINDLER, W. and HÄFLIGER, F., *Helv. Chim. Acta* **37**, 472 (1954).
92. DOMENJOZ, R. and THEOBALD, W., *Arch. Int. Pharmacodyn.* **120**, 450 (1959).
93. SIGG, E., *Canad. Psychiatric. Ass. J.*, **4**, 75 (1959).
94. RANDALL, L. and BAGDON, R., *Ann. N. Y. Acad. Sci.* **80**, 626, (1959).
95. PLETSCHER, A., GEY, K. and ZELLER, P., *Progress in Drug Research* **2**, 417 (1960).
96. HALPERN, B. and DUCROT, R., *C. Rend. Soc. Biol.* **140**, 361 (1946).
97. HALPERN, B., *C. Rend. Soc. Biol.* **140**, 363 (1946).
98. HALPERN, B. and BRIOT, M., *C. Rend. Soc. Biol.* **144**, 887 (1950).
99. HALPERN, B., *Arch. Int. Pharmacodyn.* **68**, 339 (1942).
100. SCHMITERLÖW, C., *Arch. Int. Pharmacodyn.* **108**, 431 (1956).
101. BJERVER, K. and GOLDBERG, L., *Acta Physiol. Scand. suppl.* **89**, 25, 10 (1951).
102. FÜHRER, C. and FELDHOFFEN, E., *Arzneimittelforschung* **11**, 1027 (1961).
103. GARBERG, L. and SANDBERG, F., *Acta Pharmacol. (Kobenhavn)* **16**, 367 (1960).
104. MELANDER, B. and GLINIECKE, G., *Acta Pharmacol. (Kobenhavn)* **18**, 239 (1961).
105. GUNN, J. and GURD, M., *J. Physiol.* **97**, 453 (1956).
106. CHANCE, M., *J. Pharmacol. Exp. Ther.* **87**, 214 (1946).
107. BROWN, B., *Int. Physiol. Congr. Brussels* pg. 133 (1956).
108. CARLSSON, A., *Acta Universitatis Gothoburgensis* **66**, 1 (1960).
109. DOMINO, E., *Clin. Pharmacol. Ther.* **4**, 231 (1963).
110. JONSSON, C.-O. and ANDERSÉN, K., *Svensk Lakartidn.* **57**, 1213 (1960).
111. BENSON, W. and SCHIELE, B., *Tranquilizing and antidepressive drugs*, Springfield, Illinois (1962).
112. SCHAUMANN, W. and STOEPEL, K., *Arch. exp. Path. Pharmacol.* **241**, 383 (1961).
113. LJUNGBERG, S., *Svensk Farm. T.* **66**, 445, (1962).
114. CARLSSON, A. and LINDQVIST, M., *Acta Pharmacol. (Kobenhavn)* **20**, 140 (1963).
115. BRADLEY, P., *Physiological Pharmacology*, New York (1963).
116. MASON-BROWNE, N., *Canad. Med. Ass. J.*, **79**, 985 (1958).
117. STERNER, N., to be published.

